

**INTERLEUKIN-3 (IL-3) MULTIPLE MUTATION POLYPEPTIDES**

This is a continuation-in-part of United States Application Serial No. 07/981,044 filed November 24, 1992 which is incorporated herein by reference.

Field of the Invention

The present invention relates to mutants or variants of human interleukin-3 (hIL-3) which contain multiple amino acid substitutions and which may have portions of the native hIL-3 molecule deleted. These hIL-3 multiple mutation polypeptides retain one or more activities of native hIL-3 and may also show improved hematopoietic cell-stimulating activity and/or an improved activity profile which may include reduction of undesirable biological activities associated with native hIL-3.

Background of the Invention

Colony stimulating factors (CSFs) which stimulate the differentiation and/or proliferation of bone marrow cells have generated much interest because of their therapeutic potential for restoring depressed levels of hematopoietic stem cell-derived cells. CSFs in both human and murine systems have been identified and distinguished according to their activities. For example, granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) stimulate the in vitro formation of neutrophilic granulocyte and macrophage colonies, respectively while GM-CSF and interleukin-3 (IL-3) have broader activities and stimulate the formation of both macrophage, neutrophilic and eosinophilic granulocyte colonies. IL-3 also stimulates the formation of mast, megakaryocyte and pure and mixed erythroid colonies.

Because of its ability to stimulate the

proliferation of a number of different cell types and to support the growth and proliferation of progenitor cells, IL-3 has potential for therapeutic use in restoring hematopoietic cells to normal amounts in those cases where the number of cells has been reduced due to diseases or to therapeutic treatments such as radiation and chemotherapy.

Interleukin-3 (IL-3) is a hematopoietic growth factor which has the property of being able to promote the survival, growth and differentiation of hematopoietic cells. Among the biological properties of IL-3 are the ability (a) to support the growth and differentiation of progenitor cells committed to all, or virtually all, blood cell lineages; (b) to interact with early multipotential stem cells; (c) to sustain the growth of pluripotent precursor cells; (d) to stimulate proliferation of chronic myelogenous leukemia (CML) cells; (e) to stimulate proliferation of mast cells, eosinophils and basophils; (f) to stimulate DNA synthesis by human acute myelogenous leukemia (AML) cells; (g) to prime cells for production of leukotrienes and histamines; (h) to induce leukocyte chemotaxis; and (i) to induce cell surface molecules needed for leukocyte adhesion.

Mature human interleukin-3 (hIL-3) consists of 133 amino acids. It has one disulfide bridge and two potential glycosylation sites (Yang, et al., CELL 47:3 (1986)).

Murine IL-3 (mIL-3) was first identified by Ihle, et al., J. IMMUNOL. 126:2184 (1981) as a factor which induced expression of a T cell associated enzyme, 20-hydroxysteroid dehydrogenase. The factor was purified to homogeneity and shown to regulate the growth and differentiation of numerous subclasses of early hematopoietic and lymphoid progenitor cells.

In 1984, cDNA clones coding for murine IL-3 were isolated (Fung, et al., NATURE 307:233 (1984) and Yokota, et al., PROC. NATL. ACAD. SCI. USA 81:1070 (1984)). The murine DNA sequence coded for a
 5 polypeptide of 166 amino acids including a putative signal peptide.

The gibbon IL-3 sequence was obtained using a gibbon cDNA expression library. The gibbon IL-3 sequence was then used as a probe against a human
 10 genomic library to obtain a human IL-3 sequence.

Gibbon and human genomic DNA homologues of the murine IL-3 sequence were disclosed by Yang, et al., CELL 47:3 (1986). The human sequence reported by Yang, et al. included a serine residue at position 8
 15 of the mature protein sequence. Following this finding, others reported isolation of Pro⁸ hIL-3 cDNAs having proline at position 8 of the protein sequence. Thus it appears that there may be two allelic forms of hIL-3.

Dorssers, et al., GENE 55:115 (1987), found a
 20 clone from a human cDNA library which hybridized with mIL-3. This hybridization was the result of the high degree of homology between the 3' noncoding regions of mIL-3 and hIL-3. This cDNA coded for an hIL-3 (Pro⁸)
 25 sequence.

U.S. 4,877,729 and U.S. 4,959,454 disclose human IL-3 and gibbon IL-3 cDNAs and the protein sequences for which they code. The hIL-3 disclosed has serine rather than proline at position 8 in the protein
 30 sequence.

Clark-Lewis, et al., SCIENCE 231:134 (1986) performed a functional analysis of murine IL-3 analogues synthesized with an automated peptide synthesizer. The authors concluded that the stable
 35 tertiary structure of the complete molecule was required for full activity. A study on the role of

the disulfide bridges showed that replacement of all four cysteines by alanine gave a molecule with 1/500th the activity as the native molecule. Replacement of two of the four Cys residues by Ala(Cys⁷⁹, Cys¹⁴⁰ -> Ala⁷⁹, Ala¹⁴⁰) resulted in an increased activity. The authors concluded that in murine IL-3 a single disulfide bridge is required between cysteines 17 and 80 to get biological activity that approximates physiological levels and that this structure probably stabilizes the tertiary structure of the protein to give a conformation that is optimal for function. (Clark-Lewis, et al., PROC. NATL. ACAD. SCI. USA 85:7897 (1988)).

International Patent Application (PCT) WO 88/00598 discloses gibbon- and human-like IL-3. The hIL-3 contains a Ser⁸ -> Pro⁸ replacement. Suggestions are made to replace Cys by Ser, thereby breaking the disulfide bridge, and to replace one or more amino acids at the glycosylation sites.

EP-A-0275598 (WO 88/04691) illustrates that Ala¹ can be deleted while retaining biological activity. Some mutant hIL-3 sequences are provided, e.g., two double mutants, Ala¹ -> Asp¹, Trp¹³ -> Arg¹³ (pGB/IL-302) and Ala¹ -> Asp¹, Met³ -> Thr³ (pGB/IL-304) and one triple mutant Ala¹ -> Asp¹, Leu⁹ -> Pro⁹, Trp¹³ -> Arg¹³ (pGB/IL-303).

WO 88/05469 describes how deglycosylation mutants can be obtained and suggests mutants of Arg⁵⁴Arg⁵⁵ and Arg¹⁰⁸Arg¹⁰⁹Lys¹¹⁰ might avoid proteolysis upon expression in Saccharomyces cerevisiae by KEX2 protease. No mutated proteins are disclosed. Glycosylation and the KEX2 protease activity are only important, in this context, upon expression in yeast.

WO 88/06161 mentions various mutants which theoretically may be conformationally and antigenically neutral. The only actually performed

mutations are Met² -> Ile² and Ile¹³¹ -> Leu¹³¹. It is not disclosed whether the contemplated neutralities were obtained for these two mutations.

WO 91/00350 discloses nonglycosylated hIL-3 analog proteins, for example, hIL-3 (Pro⁸Asp¹⁵Asp⁷⁰), Met³ rhIL-3 (Pro⁸Asp¹⁵Asp⁷⁰); Thr⁴ rhIL-3 (Pro⁸Asp¹⁵Asp⁷⁰) and Thr⁶ rhIL-3 (Pro⁸Asp¹⁵Asp⁷⁰). It is said that these protein compositions do not exhibit certain adverse side effects associated with native hIL-3 such as urticaria resulting from infiltration of mast cells and lymphocytes into the dermis. The disclosed analog hIL-3 proteins may have N termini at Met³, Thr⁴, or Thr⁶.

WO 91/12874 discloses cysteine added variants (CAVs) of IL-3 which have at least one Cys residue substituted for a naturally occurring amino acid residue.

Summary of the Invention

The present invention relates to recombinant human interleukin-3 (hIL-3) variant or mutant proteins (muteins). These hIL-3 muteins contain amino acid substitutions and may also have amino acid deletions at either/or both the N- and C- termini. Preferably, these mutant polypeptides of the present invention contain four or more amino acids which differ from the amino acids found at the corresponding positions in the native hIL-3 polypeptide. The invention also relates to pharmaceutical compositions containing the hIL-3 muteins, DNA coding for the muteins, and methods for using the muteins. Additionally, the present invention relates to recombinant expression vectors comprising nucleotide sequences encoding the hIL-3 muteins, related microbial expression systems, and processes for making the hIL-3 muteins using the microbial expression systems.

to bind to the IL-3 receptor complex while intrinsic activation properties of the ligand are diminished. IL-3, GM-CSF and/or IL-5 also play a role in certain asthmatic responses. An antagonist of the IL-3
 5 receptor may have the utility in this disease by blocking receptor-mediated activation and recruitment of inflammatory cells.

Brief Description of the Drawings

10 Figure 1 is the human IL-3 gene for E. coli expression (pMON5873), encoding the polypeptide sequence of natural (wild type) human IL-3 [SEQ ID NO:128], plus an initiator methionine, as expressed in E. coli, with the amino acids numbered from the N-
 15 terminus of the natural hIL-3.

Figure 2: ClaI to NsiI Replacement Fragment. Figure 2 shows the nucleotide sequence of the replacement fragment used between the ClaI and NsiI sites of the hIL-3 gene. The codon choice used in the
 20 fragment corresponds to that found in highly expressed E. coli genes (Gouy and Gautier, 1982). Three new unique restriction sites, EcoRV, XhoI and PstI were introduced for the purpose of inserting synthetic gene fragments. The portion of the coding sequence shown
 25 encodes hIL-3 amino acids 20-70.

Figure 3 shows the nucleotide and amino acid sequence of the gene in pMON5873 with the sequence extending from NcoI through HindIII. The codon choices used to encode amino acids 1-14 and 107-133
 30 correspond to that found in highly expressed E. coli genes.

Figure 4 shows the construction of the plasmid vector pMON5846 which encodes [Met-(1-133) hIL-3 (Arg¹²⁹)].

35 Figure 5 shows the construction of the plasmid vector pMON5847 (ATCC 68912) which encodes [Met-(1-

133) hIL-3 (Arg¹²⁹)].

Figure 6 shows the construction of plasmid vector pMON5853 which encodes [Met-(15-133) hIL-3 (Arg¹²⁹)].

Figure 7 shows the construction of the plasmid
5 vector pMON5854 which encodes [Met-(1-133) hIL-3 (Arg¹²⁹)].

Figure 8 shows the DNA sequence and resulting amino acid sequence of the LamB signal peptide.

Figure 9 shows the construction of the plasmid
10 vector pMON5978 which encodes Met-Ala-(15-125)hIL-3.

Figure 10 shows the construction of the plasmid vector pMON5988 which encodes Met-Ala(15-125)hIL-3.

Figure 11 shows the construction of the plasmid vector pMON5887 which encodes Met-(1-125)hIL-3.

15 Figure 12 shows the construction of pMON6457 which encodes (15-125)hIL-3; it contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 13 shows the construction of pMON6458; it
20 contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 14 shows the construction of pMON13359.

Figure 15 shows the construction of pMON13352.

Figure 16 shows the construction of pMON13360.

25 Figure 17 shows the construction of pMON13363.

Figure 18 shows the construction of pMON13364.

Figure 19 shows the construction of pMON13365.

Figure 20 shows the construction of pMON13287.

Figure 21 shows the construction of pMON13288.

30 Figure 22 shows the construction of pMON13289.

Figure 23 shows the construction of pMON5723.

Figure 24 shows the construction of pMON13438.

Detailed Description of the Invention

35 The present invention relates to muteins of human interleukin-3 (hIL-3) in which amino acid

substitutions have been made at four or more positions in amino acid sequence of the polypeptide and to muteins which have substantially the same structure and substantially the same biological activity.

5 Preferred muteins of the present invention are (15-125)hIL-3 deletion mutants which have deletions of amino acids 1 to 14 at the N-terminus and 126 to 133 at the C-terminus and which also have four or more amino acid substitutions in the polypeptide and
10 muteins having substantially the same structure and substantially the same biological activity. Among the preferred muteins are those having twenty-six amino acid substitutions. As used herein human interleukin-3 corresponds to the amino acid sequence (1-133) as
15 depicted in Figure 1 and (15-125) hIL-3 corresponds to the 15 to 125 amino acid sequence of the hIL-3 polypeptide. Naturally occurring variants of hIL-3 polypeptide amino acids are also included in the present invention (for example, the allele in which
20 proline rather than serine is at position 8 in the hIL-3 polypeptide sequence) as are variant hIL-3 molecules which are modified post-translationally (e.g. glycosylation).

The present invention also includes the DNA
25 sequences which code for the mutant polypeptides, DNA sequences which are substantially similar and perform substantially the same function, and DNA sequences which differ from the DNAs encoding the muteins of the invention only due to the degeneracy of the genetic
30 code.

Included in the present invention are novel mutant human interleukin-3 polypeptides comprising a polypeptide having the amino acid sequence of native human interleukin-3 wherein amino acids 126 to 133
35 have been deleted from the C-terminus of the native human interleukin-3 polypeptide and amino acids 1 to

14 have been deleted from the N-terminus of the native human interleukin-3 polypeptide and, in addition, polypeptides also have four or more amino acid substitutions in the polypeptide sequence.

5 Also included in the present invention are the DNA sequences coding for the muteins of the present invention; the oligonucleotide intermediates used to construct the mutant DNAs; and the polypeptides coded for by these oligonucleotides. These polypeptides may
10 be useful as antagonists or as antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols.

The mutant hIL-3 polypeptides of the present invention may also have methionine, alanine, or
15 methionine-alanine residues inserted at the N-terminus.

The present invention includes human interleukin-3 mutant polypeptide Formula I:

20

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

25

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

35

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

11

	80	85	90
	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
	95	100	105
5	Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
	110	115	120
	Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID		
10	NO:15]		
	125	130	
	wherein Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or		
	Arg;		
15	Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;		
	Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;		
	Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;		
	Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln,		
	Asn, Thr, Ser or Val;		
20	Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn,		
	Gln, Leu, Val or Gly;		
	Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe,		
	Leu, Ser, or Arg;		
	Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;		
25	Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;		
	Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;		
	Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;		
	Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;		
	Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;		
30	Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu,		
	or Lys;		
	Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;		
	Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or		
	Glu;		
35	Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;		
	Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr,		

Arg, Ala, Phe, Ile or Met;

Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;

Xaa at position 36 is Asp, Leu, or Val;

Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;

5 Xaa at position 38 is Asn, or Ala;

Xaa at position 40 is Leu, Trp, or Arg;

Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;

Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu,
Val, Glu, Phe, Tyr, Ile, Met or Ala;

10 Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys,
Gln, Arg, Thr, Gly or Ser;

Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp,
Glu, Asn, Gln, Ala or Pro;

Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys,

15 Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln,
Lys, His, Ala, Tyr, Ile, Val or Gly;

Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;

Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu,

20 Lys, Thr, Ala, Met, Val or Asn;

Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;

Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser,
Ala, Ile, Val, His, Phe, Met or Gln;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

25 Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;

Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser,
or Met;

Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn,
Lys, His, Ala or Leu;

30 Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,
Thr, Ala, Tyr, Phe, Leu, Val or Lys;

Xaa at position 57 is Asn or Gly;

Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;

35 Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;

Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;

- Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or
Val;
- 5 Xaa at position 64 is Ala, Asn, Pro, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro,
or His;
- 10 Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or
His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly,
or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
- 15 Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,
Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or
Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or
Arg;
- 20 Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,
Gln, or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly,
or Asp;
- 25 Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or
Asp;
- 30 Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or
Arg;
Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or
Lys;
- 35 Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,
His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;

- Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;
- Xaa at position 85 is Leu, Asn, Val, or Gln;
- Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;
- Xaa at position 87 is Leu, Ser, Trp, or Gly;
- 5 Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;
- Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn,
or Ser;
- Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or
Met;
- 10 Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
- Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile
or Leu;
- Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
- Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys,
- 15 His, Ala,
or Pro;
- Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr,
Asn, Lys,
Ser, Ala, Trp, Phe, Ile, or Tyr;
- 20 Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;
- Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;
- Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr,
Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
- Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln,
- 25 Gly, Ser, Phe, or His;
- Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln,
or Pro;
- Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,
Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;
- 30 Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
- Xaa at position 103 is Asp, or Ser;
- Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu,
Gln, Lys, Ala, Phe, or Gly;
- Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
- 35 Leu, Lys, Ile, Asp, or His;
- Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;

16

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa
5 20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Glu Xaa Xaa
35 40 45

10 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
15 65 70 75

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa Xaa
80 85 90

20 Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa Xaa
110 115 120

25 Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID
NO:16]
125 130

30 wherein
Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;
Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 19 is Met, Phe, Ile, Arg, or Ala;
Xaa at position 20 is Ile or Pro;
35 Xaa at position 21 is Asp or Glu;
Xaa at position 23 is Ile, Val, Ala, Leu, or Gly;

- Xaa at position 24 is Ile, Val, Phe, or Leu;
Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 26 is His, Phe, Gly, Arg, or Ala;
Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, or Val;
5 Xaa at position 29 is Gln, Asn, Leu, Arg, or Val;
Xaa at position 30 is Pro, His, Thr, Gly, or Gln;
Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 33 is Pro, Leu, Gln, Ala, or Glu;
10 Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
Glu,
Ile, Phe, Thr or Met;
Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 36 is Asp or Leu;
15 Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
Xaa at position 38 is Asn or Ala;
Xaa at position 41 is Asn, Cys, Arg, His, Met, or Pro;
Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
Met,
20 Tyr, Val or Arg;
Xaa at position 44 is Asp or Glu;
Xaa at position 45 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn,
Glu,
Ser, or Trp;
25 Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln,
Glu,
His, Ile, Lys, Tyr, Val or Gly;
Xaa at position 47 is Ile, Val, or His;
Xaa at position 49 is Met, Asn, or Asp;
30 Xaa at position 50 is Glu, Thr, Ala, Asn, Ser or Asp;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 52 is Asn or Gly;
Xaa at position 53 is Leu, Met, or Phe;
Xaa at position 54 is Arg, Ala, or Ser;
35 Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn,

- Glu, His,
 Leu, Thr, Val or Lys;
 Xaa at position 59 is Glu, Tyr, His, Leu, or Arg;
 Xaa at position 60 is Ala, Ser, Asn, or Thr;
 5 Xaa at position 61 is Phe or Ser;
 Xaa at position 62 is Asn, Val, Pro, Thr, or Ile;
 Xaa at position 63 is Arg, Tyr, Lys, Ser, His, or Val;
 Xaa at position 64 is Ala or Asn;
 Xaa at position 65 is Val, Thr, Leu, or Ser;
 10 Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
 Xaa at position 67 is Ser, Phe, Val, Gly, Asn, Ile, or His;
 Xaa at position 68 is Leu, Val, Ile, Phe, or His;
 Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
 Xaa at position 70 is Asn or Pro;
 15 Xaa at position 71 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
 Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
 Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or
 Pro;
 Xaa at position 74 is Ile or Met;
 20 Xaa at position 75 is Glu, Gly, Asp, Ser, or Gln;
 Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or
 Asp;
 Xaa at position 77 is Ile, Ser, or Leu;
 Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or
 25 Asp;
 Xaa at position 80 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
 Xaa at position 81 is Leu, or Val;
 Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,
 His,
 30 Met, Phe, Ser, Thr, Tyr or Val;
 Xaa at position 83 is Pro, Ala, Thr, Trp, or Met;
 Xaa at position 85 is Leu or Val;
 Xaa at position 87 is Leu or Ser;
 Xaa at position 88 is Ala, Arg, or Trp;
 35 Xaa at position 89 is Thr, Asp, Glu, His, Asn, or Ser;
 Xaa at position 90 is Ala, Asp, or Met;

- Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
 Xaa at position 92 is Pro or Ser;
 Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
 Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile,
 5 Phe,
 Ser or Thr;
 Xaa at position 96 is Pro or Tyr;
 Xaa at position 97 is Ile, Val, or Ala;
 Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Leu,
 10 Arg, Gln,
 Glu, Lys, Met, Ser, Tyr, Val or Pro;
 Xaa at position 99 is Ile, Leu, Val, or Phe;
 Xaa at position 100 is Lys, Leu, His, Arg, Ile, Gln, Pro, or
 Ser;
 15 Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,
 Asn, Ile, Leu or Tyr;
 Xaa at position 102 is Gly, Glu, Lys, or Ser;
 Xaa at position 104 is Trp, Val, Tyr, Met, or Leu;
 Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
 20 Leu, Lys, Ile, Asp, or His;
 Xaa at position 106 is Glu, Ser, Ala, or Gly;
 Xaa at position 108 is Arg, Ala, Gln, Ser or Lys;
 Xaa at position 109 is Arg, Thr, Glu, Leu, Ser, or Gly;
 Xaa at position 112 is Thr, Val, Gln, Glu, His, or Ser;
 25 Xaa at position 114 is Tyr or Trp;
 Xaa at position 115 is Leu or Ala;
 Xaa at position 116 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala,
 Asn,
 Gln, His, Met, Phe, Tyr or Ile;
 30 Xaa at position 117 is Thr, Ser, or Asn;
 Xaa at position 119 is Glu, Ser, Pro, Leu, Thr, or Tyr;
 Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
 Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or
 Gly;
 35 Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,
 Ile, Tyr, or Cys;

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID
NO:17]

125

130

- 5 wherein
 - Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;
 - Xaa at position 18 is Asn, His, or Ile;
 - Xaa at position 19 is Met or Ile;
 - Xaa at position 21 is Asp or Glu;
- 10 Xaa at position 23 is Ile, Ala, Leu, or Gly;
 - Xaa at position 24 is Ile, Val, or Leu;
 - Xaa at position 25 is Thr, His, Gln, or Ala;
 - Xaa at position 26 is His or Ala;
 - Xaa at position 29 is Gln, Asn, or Val;
- 15 Xaa at position 30 is Pro, Gly, or Gln;
 - Xaa at position 31 is Pro, Asp, Gly, or Gln;
 - Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
 - Xaa at position 33 is Pro or Glu;
 - Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
- 20 Glu, Ile, Phe, Thr or Met;
 - Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
 - Xaa at position 37 is Phe, Ser, Pro, or Trp;
 - Xaa at position 38 is Asn or Ala;
 - Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
- 25 Met, Tyr or Arg;
 - Xaa at position 44 is Asp or Glu;
 - Xaa at position 45 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu,
- 30 Ser or Lys;
 - Xaa at position 46 is Asp, Phe, Ser, Thr, Ala, Asn Gln, Glu, His,
- 30 Ile, Lys, Tyr, Val or Cys;
 - Xaa at position 50 is Glu, Ala, Asn, Ser or Asp;
 - Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
 - Xaa at position 54 is Arg or Ala;
 - Xaa at position 54 is Arg or Ala;
- 35 Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
 - Xaa at position 56 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu,

- Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 104 is Trp or Leu;
Xaa at position 105 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,
5 Lys, Ile, Asp, or His;
Xaa at position 106 is Glu or Gly;
Xaa at position 108 is Arg, Ala, or Ser;
Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 112 is Thr, Val, or Gln;
10 Xaa at position 114 is Tyr or Trp;
Xaa at position 115 is Leu or Ala;
Xaa at position 116 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;
Xaa at position 117 is Thr or Ser;
15 Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;
20
and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from 4 to 35 of the
25 amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3.

Included in the present invention are human interleukin-3 mutant polypeptide of the Formula IV:

30
Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15
Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa Xaa
35 20 25 30

- Xaa at position 45 is Gln, Val, Met, Leu, Ala, Asn, Glu, or Lys;
Xaa at position 46 is Asp, Phe, Ser, Gln, Glu, His, Val
or Thr;
Xaa at position 50 is Glu Asn, Ser or Asp;
5 Xaa at position 51 is Asn, Arg, Pro, Thr, or His;
Xaa at position 55 is Arg, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;
Xaa at position 62 is Asn, Pro, or Thr;
Xaa at position 64 is Ala or Asn;
10 Xaa at position 65 is Val or Thr;
Xaa at position 67 is Ser or Phe;
Xaa at position 68 is Leu or Phe;
Xaa at position 69 is Gln, Ala, Glu, or Arg;
Xaa at position 76 is Ser, Val, Asn, Pro, or Gly;
15 Xaa at position 77 is Ile or Leu;
Xaa at position 79 is Lys, Gly, Asn, Met, Arg, Ile, or Gly;
Xaa at position 80 is Asn, Gly, Glu, or Arg;
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His,
Met,
20 Phe, Ser, Thr, Tyr or Val;
Xaa at position 87 is Leu or Ser;
Xaa at position 88 is Ala or Trp;
Xaa at position 91 is Ala or Pro;
Xaa at position 93 is Thr, Asp, or Ala;
25 Xaa at position 95 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 98 is His, Ile, Asn, Ala, Thr, Gln, Glu,
Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 99 is Ile or Leu;
Xaa at position 100 is Lys or Arg;
30 Xaa at position 101 is Asp, Pro, Met, Lys, Thr, His, Pro, Asn,
Ile,
Leu or Tyr;
Xaa at position 105 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 108 is Arg, Ala, or Ser;
35 Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 112 is Thr or Gln;

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:19]

110

5

wherein

Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

10 Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn,
Thr, Ser or Val;

Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln,
Leu, Val, or Gly;

15 Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe,
Leu, Ser, or Arg;

Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

20 Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;

Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or
Lys;

25 Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;

Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr,
Arg, Ala, Phe, Ile or Met;

30 Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;

Xaa at position 22 is Asp, Leu, or Val;

Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 24 is Asn, or Ala;

Xaa at position 26 is Leu, Trp, or Arg;

35 Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;

Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr,

- Leu,
 Val, Glu, Phe, Tyr, Ile or Met;
 Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys,
 Gln,
 5 Arg, Thr, Gly or Ser;
 Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu,
 Asn, Gln, Ala or Pro;
 Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys,
 Asp,
 10 Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;
 Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln,
 Lys, His, Ala, Tyr, Ile, Val or Gly;
 Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;
 Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu,
 15 Lys, Thr, Ala, Met, Val or Asn;
 Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
 Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser,
 Ala,
 Ile, Val, His, Phe, Met or Gln;
 20 Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
 Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
 Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser,
 Met, or;
 Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn,
 25 Lys, His, Ala or Leu;
 Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;
 Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,
 Thr, Ala, Tyr, Phe, Leu, Val or Lys;
 Xaa at position 43 is Asn or Gly;
 30 Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
 Xaa at position 45 is Glu Tyr, His, Leu, Pro, or Arg;
 Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;
 Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
 Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
 35 Xaa at position 49 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
 Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;

- Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or
His;
- 5 Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or
Leu;
- Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,
10 Trp, or Asn;
- Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,
15 Gln, or Leu;
- Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or
Asp;
- Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
- 20 Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or
Asp;
- Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,
25 His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
- Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;
Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 71 is Leu, Asn, Val, or Gln;
Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;
- 30 Xaa at position 73 is Leu, Ser, Trp, or Gly;
Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or
Ser;
- Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;
35 Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile

- or Leu;
- Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
- Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His,
- 5 Ala or Pro;
- Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn,
- Lys, Ser, Ala, Trp, Phe, Ile or Tyr;
- Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;
- 10 Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;
- Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
- Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
- 15 Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, Pro;
- Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;
- Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
- 20 Xaa at position 89 is Asp, or Ser;
- Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
- Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
- 25 Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
- Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala, or Pro;
- Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
- Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg,
- 30 His, Glu, Ser, Ala or Trp;
- Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;
- Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
- Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
- 35 Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
- Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,

32

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa
5 80 85 90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa
95 100 105

10 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:20]
110

wherein

- 15 Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 5 is Met, Phe, Ile, Arg, or Ala;
Xaa at position 6 is Ile or Pro;
Xaa at position 7 is Asp, or Glu;
- 20 Xaa at position 9 is Ile, Val, Ala, Leu, or Gly;
Xaa at position 10 is Ile, Val, Phe, or Leu;
Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 12 is His, Phe, Gly, Arg, or Ala;
Xaa at position 14 is Lys, Leu, Gln, Gly, Pro, or Val;
- 25 Xaa at position 15 is Gln, Asn, Leu, Arg, or Val;
Xaa at position 16 is Pro, His, Thr, Gly, or Gln;
Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 19 is Pro, Leu, Gln, Ala, or Glu;
- 30 Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
Glu, Ile, Phe, Thr or Met;
Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 22 is Asp or Leu;
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;
- 35 Xaa at position 24 is Asn or Ala;
Xaa at position 27 is Asn, Cys, Arg, His, Met, or Pro;

- Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
Met, Tyr, or Arg;
- Xaa at position 30 is Asp, or Glu;
- Xaa at position 31 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn Glu,
5 Ser or Trp;
- Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln,
Glu, His, Ile, Lys, Tyr, Val or Gly;
- Xaa at position 33 is Ile, Val, or His;
- Xaa at position 35 is Met, Asn, or Asp;
- 10 Xaa at position 36 is Glu, Thr, Ala, Asn, Ser or Asp;
- Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- Xaa at position 38 is Asn or Gly;
- Xaa at position 39 is Leu, Met, or Phe;
- Xaa at position 40 is Arg, Ala or Ser;
- 15 Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
- Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn,
Glu, His, Leu, Thr, Val or Lys;
- Xaa at position 45 is Glu, Tyr, His, Leu, or Arg;
- Xaa at position 46 is Ala, Ser, Asn, or Thr;
- 20 Xaa at position 47 is Phe or Ser;
- Xaa at position 48 is Asn, Val, Pro, Thr, or Ile;
- Xaa at position 49 is Arg, Tyr, Lys, Ser, His, or Val;
- Xaa at position 50 is Ala or Asn;
- Xaa at position 51 is Val, Thr, Leu, or Ser;
- 25 Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
- Xaa at position 53 is Ser, Phe, Val, Gly, Asn, Ile, or His;
- Xaa at position 54 is Leu, Val, Ile, Phe, or His;
- Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
- Xaa at position 56 is Asn or Pro;
- 30 Xaa at position 57 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
- Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
- Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or
Pro;
- Xaa at position 60 is Ile or Met;
- 35 Xaa at position 61 is Glu, Gly, Asp, Ser, or Gln;
- Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or

- Asp;
- Xaa at position 63 is Ile, Ser, or Leu;
- Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;
- 5 Xaa at position 66 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
- Xaa at position 67 is Leu, or Val;
- Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
- Xaa at position 69 is Pro, Ala, Thr, Trp, or Met;
- 10 Xaa at position 71 is Leu or Val;
- Xaa at position 73 is Leu or Ser;
- Xaa at position 74 is Ala, Arg, or Trp;
- Xaa at position 75 is Thr, Asp, Glu, His, Asn, or Ser;
- Xaa at position 76 is Ala, Asp, or Met;
- 15 Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
- Xaa at position 78 is Pro or Ser;
- Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
- Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe,
- 20 Ser or Thr;
- Xaa at position 82 is Pro or Tyr;
- Xaa at position 83 is Ile, Val, or Ala;
- Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Pro;
- 25 Xaa at position 85 is Ile, Leu, Val, or Phe;
- Xaa at position 86 is Lys, Leu, His, Arg, Ile, Gln, Pro or Ser;
- Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Asn, Ile, Leu or Tyr;
- 30 Xaa at position 88 is Gly, Glu, Lys, or Ser;
- Xaa at position 90 is Trp, Val, Tyr, Met, or Leu;
- Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
- Xaa at position 92 is Glu, Ser, Ala, or Gly;
- 35 Xaa at position 94 is Arg, Ala, Gln, Ser or Lys;
- Xaa at position 95 is Arg, Thr, Glu, Leu, Ser, or Gly;

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr
65 70 75

Ala Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa
5 80 85 90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu
95 100 105

10 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:21]
110

wherein

- Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
- 15 Xaa at position 4 is Asn, His, or Ile;
- Xaa at position 5 is Met or Ile;
- Xaa at position 7 is Asp or Glu;
- Xaa at position 9 is Ile, Ala, Leu, or Gly;
- Xaa at position 10 is Ile, Val, or Leu;
- 20 Xaa at position 11 is Thr, His, Gln, or Ala;
- Xaa at position 12 is His or Ala;
- Xaa at position 15 is Gln, Asn, or Val;
- Xaa at position 16 is Pro, Gly, or Gln;
- Xaa at position 17 is Pro, Asp, Gly, or Gln;
- 25 Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
- Xaa at position 19 is Pro or Glu;
- Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg,
Gln, Glu, Ile, Phe, Thr or Met;
- Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
- 30 Xaa at position 23 is Phe, Ser, Pro, or Trp;
- Xaa at position 24 is Asn or Ala;
- Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile,
Leu, Met Tyr or Arg;
- Xaa at position 30 is Asp or Glu;
- 35 Xaa at position 31 is Gln, Val, Met, Leu, Thr, Ala, Asn,
Glu, Ser or Lys;

- Xaa at position 32 is Asp, Phe, Ser, Thr, Ala, Asn, Gln, Glu,
His, Ile, Lys, Tyr, Val or Cys;
- Xaa at position 36 is Glu, Ala, Asn, Ser or Asp;
- Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- 5 Xaa at position 40 is Arg or Ala;
- Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
- Xaa at position 42 is Pro, Gly, Ser, Gln, Ala, Arg, Asn,
Glu, Leu, Thr, Val or Lys;
- Xaa at position 46 is Ala or Ser;
- 10 Xaa at position 48 is Asn, Pro, Thr, or Ile;
- Xaa at position 49 is Arg or Lys;
- Xaa at position 50 is Ala or Asn;
- Xaa at position 51 is Val or Thr;
- Xaa at position 52 is Lys or Arg;
- 15 Xaa at position 53 is Ser, Phe, or His;
- Xaa at position 54 is Leu, Ile, Phe, or His;
- Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
- Xaa at position 57 is Ala, Pro, or Arg;
- Xaa at position 58 is Ser, Glu, Arg, or Asp;
- 20 Xaa at position 59 is Ala or Leu;
- Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;
- Xaa at position 63 is Ile or Leu;
- Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly,
or Asp;
- 25 Xaa at position 66 is Asn, Gly, Glu, or Arg;
- Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,
His, Ile, Met, Phe, Ser, Thr, Tyr or Val;
- Xaa at position 69 is Pro or Thr;
- Xaa at position 71 is Leu or Val;
- 30 Xaa at position 73 is Leu or Ser;
- Xaa at position 74 is Ala or Trp;
- Xaa at position 77 is Ala or Pro;
- Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
- Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe,
35 Ser or Thr;
- Xaa at position 82 is Pro or Tyr;

- Xaa at position 83 is Ile or Val;
Xaa at position 84 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg,
Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 85 is Ile, Leu, or Val;
5 Xaa at position 86 is Lys, Arg, Ile, Gln, Pro, or Ser;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Asn, Ile,
Leu or Tyr;
Xaa at position 90 is Trp or Leu;
Xaa at position 91 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,
10 Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, or Gly;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 98 is Thr, Val, or Gln;
15 Xaa at position 100 is Tyr or Trp;
Xaa at position 101 is Leu or Ala;
Xaa at position 102 is Lys, Thr, Val, Trp, Ser, Ala, His,
Met, Phe, Tyr or Ile;
Xaa at position 103 is Thr or Ser;
20 Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,
Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;
25 which can additionally have Met- or Met-Ala- preceding the amino
acid in position 1; and wherein from 4 to 35 of the amino acids
designated by Xaa are different from the corresponding amino
acids of native human interleukin-3.

30

Included in the present invention are (15-125)human
interleukin-3 mutant polypeptides of the Formula VIII:

35 Asn Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa
1 5 10 15

Xaa Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp
20 25 30

5 Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu
35 40 45

Ala Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile
50 55 60

10 Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr
65 70 75

Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp
15 80 85 90

Xaa Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu
95 100 105

20 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:22]
110

wherein

25 Xaa at position 3 is Ser, Gly, Asp, or Gln;
Xaa at position 4 is Asn, His, or Ile;
Xaa at position 9 is Ile, Ala, Leu, or Gly;
Xaa at position 11 is Thr, His, or Gln;
Xaa at position 12 is His or Ala;
Xaa at position 15 is Gln or Asn;

30 Xaa at position 16 is Pro or Gly;
Xaa at position 18 is Leu, Arg, Asn, or Ala;
Xaa at position 20 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,
Phe, Thr or Met;.

Xaa at position 21 is Leu, Ala, Asn, or Pro;

35 Xaa at position 24 is Asn or Ala;
Xaa at position 28 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met,

Tyr or Arg;

Xaa at position 31 is Gln, Val, Met, Leu, Ala, Asn, Glu or Lys;

Xaa at position 32 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val
or Thr;

5 Xaa at position 36 is Glu, Asn, Ser or Asp;

Xaa at position 37 is Asn, Arg, Pro, Thr, or His;

Xaa at position 41 is Arg, Leu, or Gly;

Xaa at position 42 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;

Xaa at position 48 is Asn, Pro, or Thr;

10 Xaa at position 50 is Ala or Asn;

Xaa at position 51 is Val or Thr;

Xaa at position 53 is Ser or Phe;

Xaa at position 54 is Leu or Phe;

Xaa at position 55 is Gln, Ala, Glu, or Arg;

15 Xaa at position 62 is Ser, Val, Asn, Pro, or Gly;

Xaa at position 63 is Ile or Leu;

Xaa at position 65 is Lys, Asn, Met, Arg, Ile, or Gly;

Xaa at position 66 is Asn, Gly, Glu, or Arg;

Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His,

20 Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 73 is Leu or Ser;

Xaa at position 74 is Ala or Trp;

Xaa at position 77 is Ala or Pro;

Xaa at position 79 is Thr, Asp, or Ala;

25 Xaa at position 81 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;

Xaa at position 84 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu,

Lys, Met, Ser, Tyr, Val or Leu;

Xaa at position 85 is Ile or Leu;

Xaa at position 86 is Lys or Arg;

30 Xaa at position 87 is Asp, Pro, Met, Lys, His, Pro, Asn, Ile,

Leu or Tyr;

Xaa at position 91 is Asn, Pro, Ser, Ile or Asp;

Xaa at position 94 is Arg, Ala, or Ser;

Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;

35 Xaa at position 98 is Thr or Gln;

Xaa at position 102 is Lys, Val, Trp, or Ile;

Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln Thr Thr Leu
130

Ser Leu Ala Ile Phe [SEQ ID NO:129]

- 5 wherein m is 0 or 1; Xaa at position 18 is Asn or Ile; Xaa at position 19 is Met, Ala or Ile; Xaa at position 20 is Ile, Pro or Ile; Xaa at position 23 is Ile, Ala or Leu; Xaa at position 25 is Thr or His; Xaa at position 29 is Gln, Arg, Val or Ile; Xaa at position 32 is Leu, Ala, Asn or Arg; Xaa at position 34 is Leu or Ser; Xaa at position 37 is Phe, Pro, or Ser; Xaa at position 38 is Asn or Ala; Xaa at position 42 is Gly, Ala, Ser, Asp or Asn; Xaa at position 45 is Gln, Val, or Met; Xaa at position 46 is Asp or Ser; Xaa at position 49 is Met, Ile, Leu or Asp; Xaa at position 50 is Glu or Asp; Xaa at position 51 is Asn Arg or Ser; Xaa at position 55 is Arg, Leu, or Thr; Xaa at position 56 is Pro or Ser; Xaa at position 59 is Glu or Leu; Xaa at position 60 is Ala or Ser; Xaa at position 62 is Asn, Val or Pro; Xaa at position 63 is Arg or His; Xaa at position 65 is Val or Ser; Xaa at position 67 is Ser, Asn, His or Gln; Xaa at position 69 is Gln or Glu; Xaa at position 73 is Ala or Gly; Xaa at position 76 is Ser, Ala or Pro; Xaa at position 79 is Lys, Arg or Ser; Xaa at position 82 is Leu, Glu, Val or Trp; Xaa at position 85 is Leu or Val; Xaa at position 87 is Leu, Ser, Tyr; Xaa at position 88 is Ala or Trp; Xaa at position 91 is Ala or Pro; Xaa at position 93 is Pro or Ser; Xaa at position 95 is His or Thr; Xaa at position 98 is His, Ile, or Thr; Xaa at position 100 is Lys or Arg; Xaa at position 101 is Asp, Ala or Met; Xaa at position 105 is Asn or Glu; Xaa at position 109 is Arg, Glu or Leu; Xaa at position 112 is Thr or Gln; Xaa at position 116 is Lys, Val, Trp or Ser; Xaa at position 117 is Thr or Ser; Xaa at position 120 is Asn, Gln, or His; Xaa at
- 10
- 15
- 20
- 25
- 30
- 35

position 123 is Ala or Glu; with the proviso that from
four to twenty-six of the amino acids designated by
Xaa are different from the corresponding amino acids
of native human interleukin-3; or a polypeptide having
5 substantially the same structure and substantially the
same biological activity.

Preferred polypeptides of the present invention
are those of the formula

	1		.5		10
10	(Met _m -Alan) _p -Asn	Cys	Ser	Xaa	Xaa Xaa Asp Glu Xaa Ile
	15		20		
	Xaa His Leu Lys Xaa Pro Pro Xaa Pro Xaa Leu Asp Xaa				
	25		30		35
	Xaa Asn Leu Asn Xaa Glu Asp Xaa Xaa Ile Leu Xaa Glu				
15	40		45		
	Xaa Asn Leu Arg Xaa Xaa Asn Leu Xaa Xaa Phe Xaa Xaa				
	50		55		60
	Ala Xaa Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa				
	65		70		75
20	Ile Leu Xaa Asn Xaa Xaa Pro Cys Xaa Pro Xaa Ala Thr				
	80		85		
	Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Ile Xaa Xaa Gly				
	90		95		100
	Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Xaa Phe Tyr Leu				
25	105		110		
	Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln [SEQ ID				
	NO:130]				

wherein m is 0 or 1; n is 0 or 1; p is 0 or 1; Xaa at
30 position 4 is Asn or Ile; Xaa at position 5 is Met,
Ala or Ile; Xaa at position 6 is Ile, Pro or Leu; Xaa
at position 9 is Ile, Ala or Leu; Xaa at position 11
is Thr or His; Xaa at position 15 is Gln, Arg, Val or
Ile; Xaa at position 18 is Leu, Ala, Asn or Arg; Xaa
35 at position 20 is Leu or Ser; Xaa at position 23 is
Phe, Pro, or Ser; Xaa at position 24 is Asn or Ala;

Xaa at position 28 is Gly, Ala, Ser, Asp or Asn; Xaa at position 31 is Gln, Val, or Met; Xaa at position 32 is Asp or Ser; Xaa at position 35 is Met, Ile or Asp; Xaa at position 36 is Glu or Asp; Xaa at position 37
5 is Asn, Arg or Ser; Xaa at position 41 is Arg, Leu, or Thr; Xaa at position 42 is Pro or Ser; Xaa at position 45 is Glu or Leu; Xaa at position 46 is Ala or Ser; Xaa at position 48 is Asn, Val or Pro; Xaa at position 49 is Arg or His; Xaa at position 51 is Val or Ser;
10 Xaa at position 53 is Ser, Asn, His or Gln; Xaa at position 55 is Gln or Glu; Xaa at position 59 is Ala or Gly; Xaa at position 62 is Ser, Ala or Pro; Xaa at position 65 is Lys, Arg or Ser; Xaa at position 67 is Leu, Glu, or Val; Xaa at position 68 is Leu, Glu, Val
15 or Trp; Xaa at position 71 is Leu or Val; Xaa at position 73 is Leu, Ser or Tyr; Xaa at position 74 is Ala or Trp; Xaa at position 77 is Ala or Pro; Xaa at position 79 is Pro or Ser; Xaa at position 81 is His or Thr; Xaa at position 84 is His, Ile, or Thr; Xaa at
20 position 86 is Lys or Arg; Xaa at position 87 is Asp, Ala or Met; Xaa at position 91 is Asn or Glu; Xaa at position 95 is Arg, Glu, Leu; Xaa at position 98 Thr or Gln; Xaa at position 102 is Lys, Val, Trp or Ser; Xaa at position 103 is Thr or Ser; Xaa at position 106
25 is Asn, Gln, or His; Xaa at position 109 is Ala or Glu; with the proviso that from four to twenty-six of the amino acids designated by Xaa are different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially
30 the same structure and substantially the same biological activity.

"Mutant amino acid sequence," "mutant protein" or "mutant polypeptide" refers to a polypeptide having an amino acid sequence which varies from a native
35 sequence or is encoded by a nucleotide sequence intentionally made variant from a native sequence.

"Mutant protein," "variant protein" or "muted protein" means a protein comprising a mutant amino acid sequence and includes polypeptides which differ from the amino acid sequence of native hIL-3 due to amino acid deletions, substitutions, or both. "Native sequence" refers to an amino acid or nucleic acid sequence which is identical to a wild-type or native form of a gene or protein.

Human IL-3 can be characterized by its ability to stimulate colony formation by human hematopoietic progenitor cells. The colonies formed include erythroid, granulocyte, megakaryocyte, granulocytic macrophages and mixtures thereof. Human IL-3 has demonstrated an ability to restore bone marrow function and peripheral blood cell populations to therapeutically beneficial levels in studies performed initially in primates and subsequently in humans (Gillio, A. P., et al. (1990); Ganser, A, et al. (1990); Falk, S., et al. (1991). Additional activities of hIL-3 include the ability to stimulate leukocyte migration and chemotaxis; the ability to prime human leukocytes to produce high levels of inflammatory mediators like leukotrienes and histamine; the ability to induce cell surface expression of molecules needed for leukocyte adhesion; and the ability to trigger dermal inflammatory responses and fever. Many or all of these biological activities of hIL-3 involve signal transduction and high affinity receptor binding. Mutant polypeptides of the present invention may exhibit useful properties such as having similar or greater biological activity when compared to native hIL-3 or by having improved half-life or decreased adverse side effects, or a combination of these properties. They may also be useful as antagonists. hIL-3 mutant polypeptides which have little or no activity when compared to

native hIL-3 may still be useful as antagonists, as antigens for the production of antibodies for use in immunology or immunotherapy, as genetic probes or as intermediates used to construct other useful hIL-3 muteins. Since hIL-3 functions by binding to its receptor(s) and triggering second messages resulting in competent signal transduction, hIL-3 muteins of this invention may be useful in helping to determine which specific amino acid sequences are responsible for these activities.

The novel hIL-3 mutant polypeptides of the present invention will preferably have at least one biological property of human IL-3 or of an IL-3-like growth factor and may have more than one IL-3-like biological property, or an improved property, or a reduction in an undesirable biological property of human IL-3. Some mutant polypeptides of the present invention may also exhibit an improved side effect profile. For example, they may exhibit a decrease in leukotriene release or histamine release when compared to native hIL-3 or (15-125) hIL-3. Such hIL-3 or hIL-3-like biological properties may include one or more of the following biological characteristics and in vivo and in vitro activities.

One such property is the support of the growth and differentiation of progenitor cells committed to erythroid, lymphoid, and myeloid lineages. For example, in a standard human bone marrow assay, an IL-3-like biological property is the stimulation of granulocytic type colonies, megakaryocytic type colonies, monocyte/macrophage type colonies, and erythroid bursts. Other IL-3-like properties are the interaction with early multipotential stem cells, the sustaining of the growth of pluripotent precursor cells, the ability to stimulate chronic myelogenous leukemia (CML) cell proliferation, the stimulation of

proliferation of mast cells, the ability to support the growth of various factor-dependent cell lines, and the ability to trigger immature bone marrow cell progenitors. Other biological properties of IL-3 have
5 been disclosed in the art. Human IL-3 also has some biological activities which may in some cases be undesirable, for example the ability to stimulate leukotriene release and the ability to stimulate increased histamine synthesis in spleen and bone
10 marrow cultures and in vivo.

Biological activity of hIL-3 and hIL-3 mutant proteins of the present invention is determined by DNA synthesis by human acute myelogenous leukemia cells (AML). The factor-dependent cell line AML 193 was
15 adapted for use in testing biological activity.

One object of the present invention is to provide hIL-3 muteins and hIL-3 deletion muteins with four or more amino acid substitutions in the polypeptide sequence which have similar or improved biological
20 activity in relation to native hIL-3 or native (15-125)hIL-3.

The present invention includes mutant polypeptides comprising minimally amino acids residues 15 to 118 of hIL-3 with or without additional amino
25 acid extensions to the N-terminus and/or C-terminus which further contain four or more amino acid substitutions in the amino acid sequence of the polypeptide. It has been found that the (15-125)hIL-3 mutant is more soluble than is hIL-3 when expressed in
30 the cytoplasm of E. coli, and the protein is secreted to the periplasm in E. coli at higher levels compared to native hIL-3.

When expressed in the E. coli cytoplasm, the above-mentioned mutant hIL-3 polypeptides of the
35 present invention may also be constructed with Met-Ala- at the N-terminus so that upon expression the Met

possibility of any potential antigenic effects or other possible undesirable side effects. For example, if a desired therapeutic effect can be achieved with a smaller amount of polypeptide it may be possible to
5 reduce or eliminate side effects associated with the administration of native IL-3 such as the stimulation of leukotriene and/or histamine release. The hIL-3 muteins of the present invention may also be useful in the activation of stem cells or progenitors which have
10 low receptor numbers. Pharmaceutical compositions containing (15-125) hIL-3 muteins of the present invention can be administered parenterally, intravenously, or subcutaneously.

As another aspect of the present invention, there
15 is provided a novel method for producing the novel family of human IL-3 muteins. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a vector containing a DNA sequence coding for expression of a
20 novel hIL-3 mutant polypeptide. Suitable cells or cell lines may be bacterial cells. For example, the various strains of E. coli are well-known as host cells in the field of biotechnology. Examples of such strains include E. coli strains JM101 [Yanish-Perron, et al. (1985)] and MON105 [Obukowicz, et al. (1992)].
25 Various strains of B. subtilis may also be employed in this method. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the
30 present invention.

Also suitable for use in the present invention are mammalian cells, such as Chinese hamster ovary cells (CHO). General methods for expression of foreign genes in mammalian cells are reviewed in:
35 Kaufman, R. J. (1987) High level production of proteins in mammalian cells, in Genetic Engineering,

Principles and Methods, Vol. 9, J. K. Setlow, editor, Plenum Press, New York. An expression vector is constructed in which a strong promoter capable of functioning in mammalian cells drives transcription of

5 a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant. For example, plasmids such as pcDNA I/Neo, pRc/RSV, and pRc/CMV (obtained from Invitrogen Corp., San Diego, California) can be used.

10 The eukaryotic secretion signal peptide coding region can be from the hIL-3 gene itself or it can be from another secreted mammalian protein (Bayne, M. L. et al. (1987) Proc. Natl. Acad. Sci. USA 84, 2638-2642). After construction of the vector containing the hIL-3

15 variant gene, the vector DNA is transfected into mammalian cells. Such cells can be, for example, the COS7, HeLa, BHK, CHO, or mouse L lines. The cells can be cultured, for example, in DMEM media (JRH Scientific). The hIL-3 variant secreted into the

20 media can be recovered by standard biochemical approaches following transient expression 24 - 72 hours after transfection of the cells or after establishment of stable cell lines following selection for neomycin resistance. The selection of suitable

25 mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al., U.S. Pat. No. 4,419,446. Another suitable mammalian cell line is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Where desired, insect cells may be utilized as

35 host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298

(Plenum Press 1986) and references cited therein. In addition, general methods for expression of foreign genes in insect cells using Baculovirus vectors are described in: Summers, M. D. and Smith, G. E. (1987) -

5 A manual of methods for Baculovirus vectors and insect cell culture procedures, Texas Agricultural Experiment Station Bulletin No. 1555. An expression vector is constructed comprising a Baculovirus transfer vector, in which a strong Baculovirus promoter (such as the

10 polyhedron promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant polypeptide. For example, the plasmid pVL1392 (obtained from Invitrogen Corp., San

15 Diego, California) can be used. After construction of the vector carrying the hIL-3 variant gene, two micrograms of this DNA is cotransfected with one microgram of Baculovirus DNA (see Summers & Smith, 1987) into insect cells, strain SF9. Pure recombinant

20 Baculovirus carrying the hIL-3 variant is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas). The hIL-3 variant secreted into the medium can be recovered by standard biochemical approaches.

25 Another aspect of the present invention provides plasmid DNA vectors for use in the method of expression of these novel hIL-3 muteins. These vectors contain the novel DNA sequences described above which code for the novel polypeptides of the

30 invention. Appropriate vectors which can transform microorganisms capable of expressing the hIL-3 muteins include expression vectors comprising nucleotide sequences coding for the hIL-3 muteins joined to transcriptional and translational regulatory sequences

35 which are selected according to the host cells used.

Vectors incorporating modified sequences as

described above are included in the present invention and are useful in the production of the hIL-3 mutant polypeptides. The vector employed in the method also contains selected regulatory sequences in operative
5 association with the DNA coding sequences of the invention and capable of directing the replication and expression thereof in selected host cells.

Additional details may be found in co-filed United States Patent Application Attorney docket
10 number 2713/1, which is hereby incorporated by reference in its entirety.

All references, patents or applications cited herein are incorporated by reference in their
15 entirety.

The present invention also includes the construction and expression of (15-125)human interleukin-3 muteins having four or more amino acid substitutions in secretion vectors that optimize
20 accumulation of correctly folded, active polypeptide. While many heterologous proteins have been secreted in E. coli there is still a great deal of unpredictability and limited success (Stader and Silhavy 1990). Full-length hIL-3 is such a protein,
25 where attempts to secrete the protein in E. coli resulted in low secretion levels. Secretion of the variant (15-125) hIL-3 mutant polypeptides of the present invention as a fusion with a signal peptide such as LamB results in correctly folded protein that
30 can be removed from the periplasm of E. coli by osmotic shock fractionation. This property of the variant (15-125) hIL-3 muteins allows for the direct and rapid screening for bioactivity of the secreted material in the crude osmotic shock fraction, which is
35 a significant advantage. Furthermore, it provides a means of using the (15-125)hIL-3 muteins to conduct

structure activity relationship (SAR) studies of the hIL-3 molecule. A further advantage of secretion of (15-125) hIL-3 muteins fused to the LamB signal peptide is that the secreted polypeptide has the correct N-terminal amino acid (Asn) due to the precise nature of the cleavage of the signal peptide by signal peptidase, as part of the secretion process.

The (15-125)hIL-3 muteins of the present invention may include hIL-3 polypeptides having Met-, Ala- or Met-Ala- attached to the N-terminus. When the muteins are expressed in the cytoplasm of E. coli, polypeptides with and without Met attached to the N-terminus are obtained. The methionine can in some cases be removed by methionine aminopeptidase.

Amino terminal sequences of hIL-3 muteins made in E. coli were determined using the method described by Hunkapillar et al., (1983). It was found that hIL-3 proteins made in E. coli from genes encoding Met-(15-125)hIL-3 were isolated as Met-(15-125) hIL-3. Proteins produced from genes encoding Met-Ala-(15-125) hIL-3 were produced as Ala-(15-125) hIL-3. The N-termini of proteins made in the cytoplasm of E. coli are affected by posttranslational processing by methionine aminopeptidase (Ben-Bassat et al., 1987) and possibly by other peptidases.

One method of creating the preferred hIL-3 (15-125) mutant genes is cassette mutagenesis [Wells, et al. (1985)] in which a portion of the coding sequence of hIL-3 in a plasmid is replaced with synthetic oligonucleotides that encode the desired amino acid substitutions in a portion of the gene between two restriction sites. In a similar manner amino acid substitutions could be made in the full-length hIL-3 gene, or genes encoding variants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been

deleted from the C-terminus. When properly assembled these oligonucleotides would encode hIL-3 variants with the desired amino acid substitutions and/or deletions from the N-terminus and/or C-terminus.

5 These and other mutations could be created by those skilled in the art by other mutagenesis methods including; oligonucleotide-directed mutagenesis [Zoller and Smith (1982, 1983, 1984), Smith (1985), Kunkel (1985), Taylor, et al. (1985), Deng and
10 Nickoloff (1992)] or polymerase chain reaction (PCR) techniques [Saiki, (1985)].

Pairs of complementary synthetic oligonucleotides encoding portions of the amino terminus of the hIL-3 gene can be made and annealed to each other. Such
15 pairs would have protruding ends compatible with ligation to NcoI at one end. The NcoI site would include the codon for the initiator methionine. At the other end of oligonucleotide pairs, the protruding (or blunt) ends would be compatible with a restriction
20 site that occurs within the coding sequence of the hIL-3 gene. The DNA sequence of the oligonucleotide would encode sequence for amino acids of hIL-3 with the exception of those substituted and/or deleted from the sequence.

25 The NcoI enzyme and the other restriction enzymes chosen should have recognition sites that occur only once in the DNA of the plasmid chosen. Plasmid DNA can be treated with the chosen restriction endonucleases then ligated to the annealed
30 oligonucleotides. The ligated mixtures can be used to transform competent JM101 cells to resistance to an appropriate antibiotic. Single colonies can be picked and the plasmid DNA examined by restriction analysis and/or DNA sequencing to identify plasmids with mutant
35 hIL-3 genes.

One example of a restriction enzyme which cleaves

within the coding sequence of the hIL-3 gene is ClaI whose recognition site is at codons 20 and 21. The use of ClaI to cleave the sequence of hIL-3 requires that the plasmid DNA be isolated from an E. coli strain that fails to methylate adenines in the DNA at GATC recognition sites. This is because the recognition site for ClaI, ATCGAT, occurs within the sequence GATCGAT which occurs at codons 19, 20 and 21 in the hIL-3 gene. The A in the GATC sequence is methylated in most E. coli host cells. This methylation prevents ClaI from cleaving at that particular sequence. An example of a strain that does not methylate adenines is GM48.

Interpretation of activity of single amino acid mutants in IL-3 (15-125)

As illustrated in Tables 6 and 9, there are certain positions in the IL-3 (15-125) molecule which are intolerant of substitutions, in that most or all substitutions at these positions resulted in a considerable decrease in bioactivity. There are two likely classes of such "down-mutations": mutations that affect overall protein structure, and mutations that interfere directly with the interaction between the IL-3 molecule and its receptor. Mutations affecting the three-dimensional structure of the protein will generally lie in the interior of the protein, while mutations affecting receptor binding will generally lie on the surface of the protein.

Although the three-dimensional structure of IL-3 is unknown, there are simple algorithms which can aid in the prediction of the structure. One such algorithm is the use of "helical wheels" (Kaiser, E.T. & Kezdy, F.J., Science, 223:249-255 (1984)). In this method, the presence of alpha helical protein structures can be predicted by virtue of their amphipathic nature.

Helices in globular proteins commonly have an exposed hydrophilic side and a buried hydrophobic side. As a broad generalization, in globular proteins, hydrophobic residues are present in the interior of the protein, and hydrophilic residues are present on the surface. By displaying the amino acid sequence of a protein on such a "helical wheel" it is possible to derive a model for which amino acids in alpha helices are exposed and which are buried in the core of the protein. Such an analysis of the IL-3 (15-125) molecule predicts that the following helical residues are buried in the core:

M19, I20, I23, I24, L27, L58, F61, A64, L68, A71, I74, I77, L78, L81, W104, F107, L111, Y114, L115, L118.

In addition, cysteine residues at positions 16 and 84 are linked by a disulfide bond, which is important for the overall structure or "folding" of the protein. Finally, mutations which result in a major disruption of the protein structure may be expressed at low level in the secretion system used in our study, for a variety of reasons: either because the mis-folded protein is poorly recognized by the secretion machinery of the cell; because mis-folding of the protein results in aggregation, and hence the protein cannot be readily extracted from the cells; or because the mis-folded protein is more susceptible to degradation by cellular proteases. Hence, a block in secretion may indicate which positions in the IL-3 molecule which are important for maintenance of correct protein structure.

In order to retain the activity of a variant of IL-3, it is necessary to retain both the structural

integrity of the protein, and retain the specific residues important for receptor contact. Hence it is possible to define specific amino acid residues in IL-3 (15-125) which must be retained in order to preserve biological activity.

Residues predicted to be important for interaction with the receptor: D21, E22, E43, D44, L48, R54, R94, D103, K110, F113.

10

Residues predicted to be structurally important: C16, L58, F61, A64, I74, L78, L81, C84, P86, P92, P96, F107, L111, L115, L118.

15

The hIL-3 muteins of the present invention may be useful in the treatment of diseases characterized by a decreased levels of either myeloid, erythroid, lymphoid, or megakaryocyte cells of the hematopoietic system or combinations thereof. In addition, they may be used to activate mature myeloid and/or lymphoid cells. Among conditions susceptible to treatment with the polypeptides of the present invention is leukopenia, a reduction in the number of circulating leukocytes (white cells) in the peripheral blood. Leukopenia may be induced by exposure to certain viruses or to radiation. It is often a side effect of various forms of cancer therapy, e.g., exposure to chemotherapeutic drugs and of infection or hemorrhage. Therapeutic treatment of leukopenia with these hIL-3 mutant polypeptides of the present invention may avoid undesirable side effects caused by treatment with presently available drugs.

The hIL-3 muteins of the present invention may be useful in the treatment of neutropenia and, for example, in the treatment of such conditions as aplastic anemia, cyclic neutropenia, idiopathic neutropenia, Chediak-Higashi syndrome, systemic lupus erythematosus (SLE), leukemia, myelodysplastic syndrome and myelofibrosis.

Many drugs may cause bone marrow suppression or hematopoietic deficiencies. Examples of such drugs are AZT, DDI, alkylating agents and anti-metabolites used in chemotherapy, antibiotics such as chloramphenicol, penicillin and sulfa drugs, phenothiazones, tranquilizers such as meprobamate, and diuretics. The hIL-3 muteins of the present invention may be useful in preventing or treating the bone marrow suppression or hematopoietic deficiencies which often occur in patients treated with these drugs.

Hematopoietic deficiencies may also occur as a

result of viral, microbial or parasitic infections and as a result of treatment for renal disease or renal failure, e.g., dialysis. The hIL-3 muteins of the present invention may be useful in treating such
5 hematopoietic deficiency.

The treatment of hematopoietic deficiency may include administration of the hIL-3 mutein of a pharmaceutical composition containing the hIL-3 mutein to a patient. The hIL-3 muteins of the present
10 invention may also be useful for the activation and amplification of hematopoietic precursor cells by treating these cells in vitro with the muteins of the present invention prior to injecting the cells into a patient.

Various immunodeficiencies e.g., in T and/or B lymphocytes, or immune disorders, e.g., rheumatoid arthritis, may also be beneficially affected by treatment with the hIL-3 mutant polypeptides of the present invention. Immunodeficiencies may be the
20 result of viral infections e.g. HTLV I, HTLV II, HTLV III, severe exposure to radiation, cancer therapy or the result of other medical treatment. The hIL-3 mutant polypeptides of the present invention may also be employed, alone or in combination with other
25 hematopoietins, in the treatment of other blood cell deficiencies, including thrombocytopenia (platelet deficiency), or anemia. Other uses for these novel polypeptides are in the treatment of patients recovering from bone marrow transplants in vivo and ex
30 vivo, and in the development of monoclonal and polyclonal antibodies generated by standard methods for diagnostic or therapeutic use.

Other aspects of the present invention are methods and therapeutic compositions for treating the
35 conditions referred to above. Such compositions comprise a therapeutically effective amount of one or

more of the hIL-3 muteins of the present invention in a mixture with a pharmaceutically acceptable carrier. This composition can be administered either parenterally, intravenously or subcutaneously. When
5 administered, the therapeutic composition for use in this invention is preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity,
10 stability and the like, is within the skill of the art.

The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician considering
15 various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, a daily regimen may be in the range of 0.2 - 150 µg/kg
20 of non-glycosylated IL-3 protein per kilogram of body weight. This dosage regimen is referenced to a standard level of biological activity which recognizes that native IL-3 generally possesses an EC₅₀ at or about 10 pM to 100 pM in the AML
25 proliferation assay described herein. Therefore, dosages would be adjusted relative to the activity of a given mutein vs. the activity of native (reference) IL-3 and it would not be unreasonable to note that dosage regimens may include doses as low as 0.1
30 microgram and as high as 1 milligram per kilogram of body weight per day. In addition, there may exist specific circumstances where dosages of IL-3 mutein would be adjusted higher or lower than the range of 10 - 200 micrograms per kilogram of body weight. These
35 include co-administration with other CSF or growth factors; co-administration with chemotherapeutic drugs

and/or radiation; the use of glycosylated IL-3 mutein; and various patient-related issues mentioned earlier in this section. As indicated above, the therapeutic method and compositions may also include co-

5 administration with other human factors. A non-exclusive list of other appropriate hematopoietins, CSFs and interleukins for simultaneous or serial co-administration with the polypeptides of the present invention includes GM-CSF, CSF-1, G-CSF, Meg-CSF, M-

10 CSF, erythropoietin (EPO), IL-1, IL-4, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, LIF, B-cell growth factor, B-cell differentiation factor and eosinophil differentiation factor, stem cell factor (SCF) also known as steel factor or c-kit ligand, or combinations

15 thereof. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by periodic assessment of the hematological profile, e.g., differential cell count

20 and the like.

Materials and methods for hIL-3 Mutein Expression in E. coli

Unless noted otherwise, all specialty chemicals were obtained from Sigma Co., (St. Louis, MO).

25 Restriction endonucleases, T4 poly-nucleotides kinase, E. coli DNA polymerase I large fragment (Klenow) and T4 DNA ligase were obtained from New England Biolabs (Beverly, Massachusetts).

Escherichia coli strains

30 Strain JM101: delta (pro lac), supE, thi, F'(traD36, rpoAB, lacI-Q, lacZdeltaM15) (Messing, 1979). This strain can be obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, accession number 33876.

35 MON 105 (W3110 rpoH358) is a derivative of W3110 (Bachmann, 1972) and has been assigned ATCC accession

number 55204. Strain GM48: *dam-3*, *dcm-6*, *gal*, *ara*, *lac*, *thr*, *leu*, *tonA*, *tsx* (Marinus, 1973) was used to make plasmid DNA that is not methylated at the sequence GATC.

5 Genes and plasmids

The gene used for hIL-3 production in *E. coli* was obtained from British Biotechnology Incorporated, Cambridge, England, catalogue number BBG14. This gene is carried on a pUC based plasmid designated pP0518.

10 The plasmids used for production of hIL-3 in *E. coli* contain genetic elements whose use has been described (Olins et al., 1988; Olins and Rangwala, 1990). The replicon used is that of pBR327 (Covarrubias, et al., 1981) which is maintained at a
15 copy number of about 100 in the cell (Soberon et al., 1980). A gene encoding the beta-lactamase protein is present on the plasmids. This protein confers ampicillin resistance on the cell. This resistance serves as a selectable phenotype for the presence of
20 the plasmid in the cell.

For cytoplasmic expression vectors the transcription promoter was derived from the *recA* gene of *E. coli* (Sancar et al., 1980). This promoter, designated *precA*, includes the RNA polymerase binding
25 site and the *lexA* repressor binding site (the operator). This segment of DNA provides high level transcription that is regulated even when the *recA* promoter is on a plasmid with the pBR327 origin of replication (Olins et al., 1988) incorporated herein
30 by reference.

In secretion expression plasmids the transcription promoter was derived from the *ara B*, *A*, and *D* genes of *E. coli* (Greenfield et al., 1978). This promoter is designated pAraBAD and is contained
35 on a 323 base pair *SacII*, *BglIII* restriction fragment. The *LamB* secretion leader (Wong et al., 1988, Clement

et al., 1981) was fused to the N-terminus of the hIL-3 gene at the recognition sequence for the enzyme *NcoI* (5'CCATGG3'). The hIL-3 genes used were engineered to have a *HindIII* recognition site (5'AAGCTT3') following the coding sequence of the gene.

These hIL-3 variants were expressed as a fusion with the LamB signal peptide shown in Figure 8, operatively joined to the araBAD promoter (Greenfield, 1978) and the gl0-L ribosome binding site (Olins et al. 1988). The processed form was selectively released from the periplasm by osmotic shock as a correctly folded and fully active molecule. Secretion of (15-125) hIL-3 was further optimized by using low inducer (arabinose) concentration and by growth at 30°C. These conditions resulted in lower accumulation levels of unprocessed LamB signal peptide (15-125) hIL-3 fusion, maximal accumulation levels of processed (15-125) hIL-3 and selective release of (15-125) hIL-3 by osmotic shock fractionation. The use of a tightly regulated promoter such as araBAD from which the transcription level and hence the expression level can be modulated allowed for the optimization of secretion of (15-125) hIL-3.

The ribosome binding site used is that from gene 10 of phage T7 (Olins et al., 1988). This is encoded in a 100 base pair (bp) fragment placed adjacent to *precA*. In the plasmids used herein, the recognition sequence for the enzyme *NcoI* (CCATGG) follows the gl0-L. It is at this *NcoI* site that the hIL-3 genes are joined to the plasmid. It is expected that the nucleotide sequence at this junction will be recognized in mRNA as a functional start site for translation (Olins et al., 1988). The hIL-3 genes used were engineered to have a *HindIII* recognition site (AAGCTT) downstream from the coding sequence of the gene. At this *HindIII* site is a 514 base pair

RsaI fragment containing the origin of replication of the single stranded phage f1 (Dente et al., 1983; Olins, et al., 1990) both incorporated herein by reference. A plasmid containing these elements is pMON2341. Another plasmid containing these elements is pMON5847 which has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 under the accession number ATCC 68912.

10 Synthesis of Oligonucleotides

Oligonucleotides were synthesized on Nucleotide Synthesizer model 380A or 380B from Applied Biosystems, Inc. (Foster City, California). Oligonucleotides were purified by polyacrylamide gel electrophoresis at concentrations from 12 - 20% (19:1 crosslinked) in 0.5 x Tris borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA) followed by passage through a Nensorb column obtained from New England Nuclear (Boston, Massachusetts) using a PREP Automated Sample Processor obtained from DuPont, Co. (Wilmington, Delaware).

Quantitation of synthetic oligonucleotides

Synthetic oligonucleotides were resuspended in water and quantitated by reading the absorbance at 260nm on a Beckman DU40 Spectrophotometer (Irvine, California) using a one centimeter by one millimeter quartz cuvette (Maniatis, 1982). The concentration was determined using an extinction coefficient of 1×10^4 (Voet et al., 1963; Mahler and Cordes, 1966). The oligonucleotides were then diluted to a desired concentration.

Quantitation of synthetic DNA fragments can also be achieved by adding 10 to 100 picomoles of DNA to a solution containing kinase buffer (25 mM Tris pH 8.0, 10 mM MgCl₂, 10 mM DTT and 2 mM spermidine). To the reaction mix is added ATP to 20 micromolar, ATP

radiolabeled at the gamma phosphate (5000-10,000 dpm/pmol) and 5 units of T4 polynucleotide kinase. Radiolabelled material is obtained from New England Nuclear (Boston, Massachusetts). The 10 microliter mixture is incubated at 37°C for one hour. A 1 microliter aliquot of the mixture was chromatographed on DEAE paper (Whatman) in 0.3 M ammonium bicarbonate. The counts that remained at the origin were used to determine the concentration of the synthetic DNA.

Recombinant DNA methods

Isolation of plasmid DNA from *E. coli* cultures was performed as described (Birnboim and Doly, 1979). Some DNAs were purified by Magic™ columns, available from Promega (Madison, Wisconsin).

Purified plasmid DNA was treated with restriction endonucleases according to manufacturer's instructions. Analysis of the DNA fragments produced by treatment with restriction enzymes was done by agarose or polyacrylamide gel electrophoresis. Agarose (DNA grade from Fisher, Pittsburgh PA.) was used at a concentration of 1.0% in a Tris-acetate running buffer (0.04 M Tris-acetate, 0.001M EDTA). Polyacrylamide (BioRad, Richmond CA.) was used at a concentration of 6% (19:1 crosslinked) in 0.5 X Tris-borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA), hereafter referred to as PAGE.

DNA polymerase I, large fragment, Klenow enzyme was used according to manufacturers instructions to catalyze the addition of mononucleotides from 5' to 3' of DNA fragments which had been treated with restriction enzymes that leave protruding ends. The reactions were incubated at 65°C for 10 minutes to heat inactivate the Klenow enzyme.

The synthetic oligonucleotides were made without 5' or 3' terminal phosphates. In cases where such

oligonucleotides were ligated end to end, the oligonucleotides were treated at a concentration of 10 picomoles per microliter with T4 polynucleotide kinase in the following buffer: 25 mM Tris, pH 8.0, 10 mM MgCl₂, 10 mM dithiothreitol, 2 mM spermidine, 1 mM rATP. After incubation for 30 minutes at 37°C, the samples were incubated at 65°C for five minutes to heat inactivate the kinase.

Synthetic gene assembly

10 The (15-125) hIL-3 gene was divided into four regions separated by five convenient restriction sites. In each of the four regions synthetic oligonucleotides were designed so that they would anneal in complementary pairs, with protruding single stranded ends, and when the pairs were properly assembled would result in a DNA sequence that encoded a portion of the hIL-3 gene. Amino acid substitutions in the hIL-3 gene were made by designing the oligonucleotides to encode the desired substitutions.

15 The complementary oligonucleotides were annealed at concentration of 1 picomole per microliter in ligation buffer plus 50mM NaCl. The samples were heated in a 100 ml beaker of boiling water and permitted to cool slowly to room temperature. One picomole of each of the annealed pairs of oligonucleotides were ligated with approximately 0.2 picomoles of plasmid DNA, digested with the appropriate restriction enzymes, in ligation buffer (25 mM Tris pH 8.0, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP, 2mM spermidine) with T4 DNA ligase obtained from New England Biolabs (Beverly, Massachusetts) in a total volume of 20 µl at room temperature overnight.

20 DNA fragments were isolated from agarose gels by intercepting the restriction fragments on DEAE membranes from Schleicher and Schuell (Keene, New Hampshire) and eluting the DNA in 10 mM Tris, 1 mM

25

30

EDTA, 1 M NaCl at 55°C for 1 hour, according to manufacturer's directions. The solutions containing the DNA fragment were concentrated and desalted by using Centricon 30 concentrators from Amicon (W.R. Grace, Beverly MA.) according to the manufacturer's directions. Ligations were performed at 15°C overnight, except as noted, in ligation buffer.

Polymerase Chain Reaction

Polymerase Chain Reaction (hereafter referred to as PCR) techniques (Saiki, 1985) used the reagent kit and thermal cycler from Perkin-Elmer Cetus (Norwalk, CT.). PCR is based on a thermostable DNA polymerase from *Thermus aquaticus*. The PCR technique is a DNA amplification method that mimics the natural DNA replication process in that the number of DNA molecules doubles after each cycle, in a way similar to in vivo replication. The DNA polymerase mediated extension is in a 5' to 3' direction. The term "primer" as used herein refers to an oligonucleotide sequence that provides an end to which the DNA polymerase can add nucleotides that are complementary to a nucleotide sequence. The latter nucleotide sequence is referred to as the "template", to which the primers are annealed. The amplified PCR product is defined as the region comprised between the 5' ends of the extension primers. Since the primers have defined sequences, the product will have discrete ends, corresponding to the primer sequences. The primer extension reaction was carried out using 20 picomoles (pmoles) of each of the oligonucleotides and 1 picogram of template plasmid DNA for 35 cycles (1 cycle is defined as 94 degrees C for one minute, 50 degrees C for two minutes and 72 degrees for three minutes.). The reaction mixture was extracted with an equal volume of phenol/chloroform (50% phenol and 50%

E. coli JM101 cells were made competent to take up DNA. Typically, 20 to 100 ml of cells were grown in LB medium to a density of approximately 150 Klett units and then collected by centrifugation. The cells were resuspended in one half culture volume of 50 mM CaCl₂ and held at 4°C for one hour. The cells were again collected by centrifugation and resuspended in one tenth culture volume of 50 mM CaCl₂. DNA was

added to a 150 microliter volume of these cells, and the samples were held at 4°C for 30 minutes. The samples were shifted to 42°C for one minute, one milliliter of LB was added, and the samples were
 5 shaken at 37°C for one hour. Cells from these samples were spread on plates containing ampicillin to select for transformants. The plates were incubated overnight at 37°C. Single colonies were picked, grown in LB supplemented with ampicillin overnight at 37°C
 10 with shaking. From these cultures DNA was isolated for restriction analysis.

Culture medium

LB medium (Maniatis et al., 1982) was used for
 15 growth of cells for DNA isolation. M9 minimal medium supplemented with 1.0% casamino acids, acid hydrolyzed casein, Difco (Detroit, Michigan) was used for cultures in which recombinant hIL-3 was produced. The ingredients in the M9 medium were as follows:
 20 3g/liter KH₂PO₄, 6g/l Na₂HPO₄, 0.5 g/l NaCl, 1 g/l NH₄Cl, 1.2 mM MgSO₄, 0.025 mM CaCl₂, 0.2% glucose (0.2% glycerol with the AraBAD promoter), 1% casamino acids, 0.1 ml/l trace minerals (per liter 108 g FeCl₃·6H₂O, 4.0 g ZnSO₄·7H₂O, 7.0 CoCl₂·2H₂O, 7.0 g
 25 Na₂MoO₄·2H₂O, 8.0 g CuSO₄·5H₂O, 2.0 g H₃BO₃, 5.0 g MnSO₄·H₂O, 100 ml concentrated HCl). Bacto agar was used for solid media and ampicillin was added to both liquid and solid LB media at 200 micrograms per milliliter.

30

DNA sequence analysis

The nucleotide sequencing of plasmid DNA was determined using a Genesis 2000 sequencer obtained from DuPont (Wilmington, Delaware) according to the
 35 methods of Prober et al. (1987) and Sanger et al. (1977). Some DNA sequences were performed using

Sequenase™ polymerase (U.S. Biochemicals, Cleveland, Ohio) according to manufacturer's directions.

Production of recombinant hIL-3 muteins in *E. coli*
5 with vectors employing the *recA* promoter

E. coli strains harboring the plasmids of interest were grown at 37°C in M9 plus casamino acids medium with shaking in a Gyrotory water bath Model G76 from New Brunswick Scientific (Edison, New Jersey).
10 Growth was monitored with a Klett Summerson meter (green 54 filter), Klett Mfg. Co. (New York, New York). At a Klett value of approximately 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining
15 culture, nalidixic acid (10mg/ml) in 0.1 N NaOH was added to a final concentration of 50 µg/ml. The cultures were shaken at 37°C for three to four hours after addition of nalidixic acid. A high degree of aeration was maintained throughout the bacterial
20 growth in order to achieve maximal production of the desired gene product. The cells were examined under a light microscope for the presence of refractile bodies (RBs). One milliliter aliquots of the culture were removed for analysis of protein content.

25 Production of recombinant hIL-3 proteins from the *pAraBAD* promoter in *E. coli*

E. coli strains harboring the plasmids of interest were grown at 30°C with shaking in M9 medium plus casamino acids and glycerol. Growth was
30 monitored with a Klett Summerson colorimeter, using a green 54 filter. At a Klett value of about 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, 20% arabinose was added to a final
35 concentration of 0.05%. The cultures were shaken at 30°C for three to four hours after addition of

arabinose. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product. One milliliter aliquots of the culture were removed for
5 analysis of protein content.

Secretion and osmotic shock

Three hour post induction samples were fractionated by osmotic shock [Neu and Heppel (1965)]. The optical density (Klett value) of the cultures was
10 determined and 1 ml of cells were centrifuged in a Sigma microcentrifuge (West Germany) model 202MK in 1.5 mls snap top microcentrifuge tubes for 5 minutes at 10,000 rpm. The cell pellet was resuspended very gently by pipeting in a room temperature sucrose
15 solution (20% sucrose w/v, 30mM Tris-Hcl pH7.5, 1mM EDTA), using 1 μ l/1 Klett unit. Following a 10 minute incubation at room temperature, the cells were centrifuged for 5 minutes at 10,000 rpm. The sucrose fraction was carefully removed from the cell pellet.
20 The cell pellet was then resuspended very gently by pipeting in ice cold distilled water, using 1 μ l/1 Klett unit. Following a 10 minute incubation on ice, the cells were centrifuged for 5 minutes at 12,000 rpm. The water fraction was carefully removed. Equal
25 volumes of the sucrose and water fractions were pooled and aliquoted to provide samples for activity screening.

Analysis of protein content of E. coli cultures producing hIL-3 mutant polypeptides

30 Bacterial cells from cultures treated as described above were collected from the medium by centrifugation. Aliquots of these cells were resuspended in SDS loading buffer (4X: 6 g SDS, 10 ml beta-mercaptoethanol, 25 ml upper Tris gel stock (0.5
35 M Tris HCl pH 6.8, 0.4% SDS) brought to 50 ml with glycerol, 0.2% bromophenol blue was added) at a

concentration of one microliter per Klett unit. These samples were incubated at 85°C for five minutes and vortexed. Five or ten microliter aliquots of these samples were loaded on 15% polyacrylamide gels prepared according to the method of Laemmli (1970). Protein bands were visualized by staining the gels with a solution of acetic acid, methanol and water at 5:1:5 ratio (volume to volume) to which Coomassie blue had been added to a final concentration of 1%. After staining, the gels were washed in the same solution without the Coomassie blue and then washed with a solution of 7% acetic acid, 5% methanol. Gels were dried on a gel drier Model SE1160 obtained from Hoeffer (San Francisco, California). The amount of stained protein was measured using a densitometer obtained from Joyce-Loebl (Gateshead, England). The values obtained were a measure of the amount of the stained hIL-3 protein compared to the total of the stained protein of the bacterial cells.

20 Western blot analysis of hIL-3 muteins made in E. coli

In some E. coli cultures producing hIL-3, the level of accumulation of the hIL-3 protein is lower than 5% of total bacterial protein. To detect hIL-3 produced at this level, Western blot analysis was used. Proteins from cultures induced with nalidixic acid or arabinose were run on polyacrylamide gels as described above except that volumes of sample loaded were adjusted to produce appropriate signals. After electrophoresis, the proteins were electroblotted to APT paper, Transa-bind, Schleicher and Schuell (Keene, New Hampshire) according to the method of Renart et al. (1979). Antisera used to probe these blots had been raised in rabbits, using peptides of the sequence of amino acids 20 to 41 and 94 to 118 of hIL-3 as the immunogens. The presence of bound antibody was detected with Staphylococcal protein A radiolabeled

with ^{125}I , obtained from New England Nuclear (Boston, Massachusetts).

Fractionation of *E. coli* cells producing hIL-3 proteins in the cytoplasm

5 Cells from *E. coli* cultures harboring plasmids that produce hIL-3 muteins were induced with nalidixic acid. After three hours, the hIL-3 muteins accumulated in refractile bodies. The first step in purification of the hIL-3 muteins was to sonicate
10 cells. Aliquots of the culture were resuspended from cell pellets in sonication buffer: 10 mM Tris, pH 8.0, 1 mM EDTA, 50 mM NaCl and 0.1 mM PMSF. These resuspended cells were subjected to several repeated sonication bursts using the microtip from a Sonicator
15 cell disrupter, Model W-375 obtained from Heat Systems-Ultrasonics Inc. (Farmingdale, New York). The extent of sonication was monitored by examining the homogenates under a light microscope. When nearly all of the cells had been broken, the homogenates were
20 fractionated by centrifugation. The pellets, which contain most of the refractile bodies, are highly enriched for hIL-3 muteins.

Methods: Extraction, Refolding and Purification of Interleukin-3 (IL-3) Muteins Expressed as Refractile
25 Bodies in *E. coli*.

Extraction of refractile bodies (RB's):

For each gram of RB's (and typically one gram is obtained from a 300 ml *E. coli* culture), 5 ml of a solution containing 6M guanidine hydrochloride
30 (GnHCl), 50 mM 2-N-cyclohexylaminoethanesulfonic acid (CHES) pH 9.5 and 20 mM dithiothreitol (DTT) was added. The RB's were extracted with a Bio-Homogenizer for 15-30 seconds and gently rocked for 2 hours at 5 degrees centigrade (5°C) to allow the protein to
35 completely reduce and denature.

Refolding of the IL-3 muteins

The protein solution was transferred to dialysis tubing (1000 molecular weight cut-off) and dialyzed against at least 100 volumes of 4M GnHCl - 50 mM CHES pH 8.0. The dialysis was continued overnight at 5°C while gently stirring. Subsequently dialysis was continued against at least 100 volumes of 2M GnHCl - 50 mM CHES pH 8.0 and dialyzed overnight at 5°C while gently stirring.

Purification of the IL-3 muteins

10 The protein solution was removed from the dialysis tubing and acidified by the addition of 40% acetonitrile (CH₃CN) - 0.2% trifluoroacetic acid (TFA) to a final concentration of 20% CH₃CN - 0.1% TFA. This was centrifuged (16,000 x g for 5 minutes) to clarify and the supernatant was loaded onto a Vydac C-18 reversed phase column (10x250 mm) available from Vydac (Hesperia, California) previously equilibrated in 20% CH₃CN - 0.1% TFA. The column was eluted with a linear gradient (0.2% CH₃CN/minute) between 40 - 50% 20 CH₃CN - 0.1% TFA at a flow rate of 3 ml/minute while collecting 1.5 ml fractions. The fractions were analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and the appropriate fractions pooled. The pooled material was dried by lyophilization or in a 25 Speed Vac concentrator. The dry powder was reconstituted with 10 mM ammonium bicarbonate pH 7.5, centrifuged (16,000 x g for 5 minutes) to clarify and assayed for protein concentration by the method of Bradford (1976) with bovine serum albumin as the 30 standard. Such protein can be further analyzed by additional techniques such as, SDS-PAGE, electrospray mass spectrometry, reverse phase HPLC, capillary zone electrophoresis, amino acid composition analysis, and ELISA (enzyme-linked immunosorbent assay).

35 hIL-3 SANDWICH ELISA

IL-3 protein concentrations can be determined using a sandwich ELISA based on an affinity purified polyclonal goat anti-rhIL-3. Microtiter plates (Dynatech Immulon II) were coated with 150 μ l goat-
5 anti-rhIL-3 at a concentration of approximately 1 μ g/ml in 100 mM NaHCO₃, pH 8.2. Plates were incubated overnight at room temperature in a chamber maintaining 100% humidity. Wells were emptied and the remaining
10 reactive sites on the plate were blocked with 200 μ l of solution containing 10 mM PBS, 3% BSA and 0.05% Tween 20, pH 7.4 for 1 hour at 37° C and 100% humidity. Wells were emptied and washed 4X with 150 mM NaCl containing 0.05% Tween 20 (wash buffer). Each well then received 150 μ l of dilution buffer (10 mM
15 PBS containing 0.1% BSA, 0.01% Tween 20, pH 7.4), containing rhIL-3 standard, control, sample or dilution buffer alone. A standard curve was prepared with concentrations ranging from 0.125 ng/ml to 5 ng/ml using a stock solution of rhIL-3 (concentration
20 determined by amino acid composition analysis). Plates were incubated 2.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 μ l of an optimal dilution (as determined in a
25 checkerboard assay format) of goat anti-rhIL-3 conjugated to horseradish peroxidase. Plates were incubated 1.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 μ l of ABTS
30 substrate solution (Kirkegaard and Perry). Plates were incubated at room temperature until the color of the standard wells containing 5 ng/ml rhIL-3 had developed enough to yield an absorbance between 0.5-1.0 when read at a test wavelength of 410 nm and a
35 reference wavelength of 570 nm on a Dynatech microtiter plate reader. Concentrations of

immunoreactive rhIL-3 in unknown samples were calculated from the standard curve using software supplied with the plate reader.

5 AML Proliferation Assay for Bioactive Human Interleukin-3

 The factor-dependent cell line AML 193 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This cell line, established
10 from a patient with acute myelogenous leukemia, is a growth factor dependent cell line which displayed enhanced growth in GM/CSF supplemented medium (Lange, B., et al., (1987); Valtieri, M., et al., (1987). The ability of AML 193 cells to proliferate
15 in the presence of human IL-3 has also been documented. (Santoli, D., et al., (1987)). A cell line variant was used, AML 193 1.3, which was adapted for long term growth in IL-3 by washing out the growth factors and starving the cytokine dependent AML 193
20 cells for growth factors for 24 hours. The cells were then replated at 1×10^5 cells/well in a 24 well plate in media containing 100 U/ml IL-3. It took approximately 2 months for the cells to grow rapidly in IL-3. These cells were maintained as AML 193 1.3
25 thereafter by supplementing tissue culture medium (see below) with human IL-3.

 AML 193 1.3 cells were washed 6 times in cold Hanks balanced salt solution (HBSS, Gibco, Grand
30 Island, NY) by centrifuging cell suspensions at $250 \times g$ for 10 minutes followed by decantation of supernatant. Pelleted cells were resuspended in HBSS and the procedure was repeated until six wash cycles were completed. Cells washed six times by this procedure were resuspended in tissue culture medium at
35 a density ranging from 2×10^5 to 5×10^5 viable cells/ml. This medium was prepared by supplementing

Iscove's modified Dulbecco's Medium (IMDM, Hazleton, Lenexa, KS) with albumin, transferrin, lipids and 2-mercaptoethanol. Bovine albumin (Boehringer-Mannheim, Indianapolis, IN) was added at 500 µg/ml; human

5 transferrin (Boehringer-Mannheim, Indianapolis, IN) was added at 100 µg/ml; soybean lipid (Boehringer-Mannheim, Indianapolis, IN) was added at 50 µg/ml; and 2-mercaptoethanol (Sigma, St. Louis, MO) was added at 5×10^{-5} M.

10 Serial dilutions of human interleukin-3 or human interleukin-3 variant protein (hIL-3 mutein) were made in triplicate series in tissue culture medium supplemented as stated above in 96 well Costar 3596 tissue culture plates. Each well contained 50 µl of

15 medium containing interleukin-3 or interleukin-3 variant protein once serial dilutions were completed. Control wells contained tissue culture medium alone (negative control). AML 193 1.3 cell suspensions prepared as above were added to each well by pipetting

20 50 µl (2.5×10^4 cells) into each well. Tissue culture plates were incubated at 37°C with 5% CO₂ in humidified air for 3 days. On day 3, 0.5 µCi ³H-thymidine (2 Ci/mM, New England Nuclear, Boston, MA) was added in 50 µl of tissue culture medium. Cultures

25 were incubated at 37°C with 5% CO₂ in humidified air for 18-24 hours. Cellular DNA was harvested onto glass filter mats (Pharmacia LKB, Gaithersburg, MD) using a TOMTEC cell harvester (TOMTEC, Orange, CT) which utilized a water wash cycle followed by a 70%

30 ethanol wash cycle. Filter mats were allowed to air dry and then placed into sample bags to which scintillation fluid (Scintiverse II, Fisher Scientific, St. Louis, MO or BetaPlate Scintillation Fluid, Pharmacia LKB, Gaithersburg, MD) was added.

35 Beta emissions of samples from individual tissue culture wells were counted in a LKB Betaplate model

1205 scintillation counter (Pharmacia LKB,
Gaithersburg, MD) and data was expressed as counts per
minute of ³H-thymidine incorporated into cells from
each tissue culture well. Activity of each human
5 interleukin-3 preparation or human interleukin-3
variant preparation was quantitated by measuring cell
proliferation (³H-thymidine incorporation) induced by
graded concentrations of interleukin-3 or interleukin-
3 variant. Typically, concentration ranges from 0.05
10 pM - 10⁵ pM are quantitated in these assays. Activity
is determined by measuring the dose of interleukin-3
or interleukin-3 variant which provides 50% of maximal
proliferation [EC₅₀ = 0.5 x (maximum average counts
per minute of ³H-thymidine incorporated per well among
15 triplicate cultures of all concentrations of
interleukin-3 tested - background proliferation
measured by ³H-thymidine incorporation observed in
triplicate cultures lacking interleukin-3]. This EC₅₀
value is also equivalent to 1 unit of bioactivity.
20 Every assay was performed with native interleukin-3 as
a reference standard so that relative activity levels
could be assigned.

Relative biological activities of IL-3 muteins of
25 the present invention are shown in Table 1. The
Relative Biological Activity of IL-3 mutants is
calculated by dividing the EC₅₀ of (1-133) hIL-3 by
the EC₅₀ of the mutant. The Relative Biological
Activity may be the average of replicate assays.

30

TABLE 1

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

35 Relative*
Plasmid

Polypeptide

Biological

Code		Structure	
Activity		Reference (1-133)hIL-3	
1			
5	pMON13298	SEQ ID NO. 82	3
	pMON13299	SEQ ID NO. 83	2
	pMON13300	SEQ ID NO. 84	3
	pMON13301	SEQ ID NO. 85	2
10	pMON13302	SEQ ID NO. 86	1.2
	pMON13303	SEQ ID NO. 87	0.6
	pMON13287	SEQ ID NO. 88	26
	pMON13288	SEQ ID NO. 89	24
	pMON13289	SEQ ID NO. 90	13
15	pMON13290	SEQ ID NO. 91	20
	pMON13292	SEQ ID NO. 92	6
	pMON13294	SEQ ID NO. 93	3
	pMON13295	SEQ ID NO. 94	3
	pMON13312	SEQ ID NO. 95	4
20	pMON13313	SEQ ID NO. 96	8
	pMON13285	SEQ ID NO. 259	32
	pMON13286	SEQ ID NO. 260	8
	pMON13325	SEQ ID NO. 261	8
	pMON13326	SEQ ID NO. 262	25
25	pMON13330	SEQ ID NO. 263	19
	pMON13329	SEQ ID NO. 406	10
	pMON13364	SEQ ID NO. 117	13

TABLE 1 (cont'd)

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

5 Relative*			
Plasmid		Polypeptide	
Biological			
Code	Structure	Activity	
10	pMON13475	SEQ ID NO. 280	7
	pMON13366	SEQ ID NO. 281	38
	pMON13367	SEQ ID NO. 282	36
	pMON13368	SEQ ID NO. 278	1.6
	pMON13369	SEQ ID NO. 283	10
15	pMON13370	SEQ ID NO. 284	6
	pMON13373	SEQ ID NO. 285	12
	pMON13374	SEQ ID NO. 286	6
	pMON13375	SEQ ID NO. 287	14
	pMON13376	SEQ ID NO. 288	0.4
20	pMON13377	SEQ ID NO. 289	0.4
	pMON13379	SEQ ID NO. 291	0.9
	pMON13380	SEQ ID NO. 279	0.05
	pMON13381	SEQ ID NO. 293	10
	pMON13382	SEQ ID NO. 313	38
25	pMON13383	SEQ ID NO. 294	0.5
	pMON13384	SEQ ID NO. 295	0.25
	pMON13385	SEQ ID NO. 292	1
	pMON13387	SEQ ID NO. 308	32
	pMON13388	SEQ ID NO. 296	23
30	pMON13389	SEQ ID NO. 297	10
	pMON13391	SEQ ID NO. 298	30
	pMON13392	SEQ ID NO. 299	17
	pMON13393	SEQ ID NO. 300	32
	pMON13394	SEQ ID NO. 301	20
35	pMON13395	SEQ ID NO. 302	11
	pMON13396	SEQ ID NO. 303	20
	pMON13397	SEQ ID NO. 304	16
	pMON13398	SEQ ID NO. 305	36
	pMON13399	SEQ ID NO. 306	18
40	pMON13404	SEQ ID NO. 307	1.3
	pMON13417	SEQ ID NO. 310	24
	pMON13420	SEQ ID NO. 311	19
	pMON13421	SEQ ID NO. 312	0.5
	pMON13432	SEQ ID NO. 313	10
45	pMON13400	SEQ ID NO. 317	0.09
	pMON13402	SEQ ID NO. 318	20
	pMON13403	SEQ ID NO. 321	0.03
	pMON13405	SEQ ID NO. 267	9

mediated sulfidoleukotriene release from human mononuclear cells.

IL-3 mediated sulfidoleukotriene release from human
5 mononuclear cells

Heparin-containing human blood was collected and layered onto an equal volume of Ficoll-Paque (Pharmacia # 17-0840-02) ready to use medium (density 1.077 g/ml.). The Ficoll was warmed to room temperature prior to use and clear 50 ml polystyrene tubes were utilized. The Ficoll gradient was spun at 300 x g for 30 minutes at room temperature using a H1000B rotor in a Sorvall RT6000B refrigerated centrifuge. The band containing the mononuclear cells was carefully removed, the volume adjusted to 50 mls with Dulbecco's phosphate-buffered saline (Gibco Laboratories cat. # 310-4040PK), spun at 400 x g for 10 minutes at 4°C and the supernatant was carefully removed. The cell pellet was washed twice with HA Buffer [20 mM Hepes (Sigma # H-3375), 125 mM NaCl (Fisher # S271-500), 5 mM KCl (Sigma # P-9541), 0.5 mM glucose (Sigma # G-5000), 0.025% Human Serum Albumin (Calbiochem # 126654) and spun at 300 x g, 10 min., 4°C. The cells were resuspended in HACM Buffer (HA buffer supplemented with 1 mM CaCl₂ (Fisher # C79-500) and 1 mM MgCl₂ (Fisher # M-33) at a concentration of 1 x 10⁶ cells/ml and 180 µl were transferred into each well of 96 well tissue culture plates. The cells were allowed to acclimate at 37°C for 15 minutes. The cells were primed by adding 10 µls of a 20 X stock of various concentrations of cytokine to each well (typically 100000, 20000, 4000, 800, 160, 32, 6.4, 1.28, 0 fM IL3). The cells were incubated for 15 minutes at 37°C. Sulfidoleukotriene release was activated by the addition of 10 µls of 20 X (1000 nM)

DNA sequences which encode mutant polypeptides of the present invention.

TABLE 2
OLIGONUCLEOTIDES

5

Oligo #1 Length: 000040

CATGGCTAAC TGCTCTATAA TGATCGATGA AATTATACAT [SEQ ID NO:15]

10

Oligo #2 Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATCAT TATAGAGCAG TTAGC [SEQ ID NO:16]

15

Oligo #3 Length: 000036

CACTTAAAGA GACCACCTGC ACCTTTGCTG GACCCG [SEQ ID NO:17]

Oligo #4 Length: 000036

GAGGTTGTTC GGGTCCAGCA AAGGTGCAGG TGGTCT [SEQ ID NO:18]

20

Oligo #5 Length: 000036

CACTTAAAGA GACCACCTAA CCCTTTGCTG GACCCG [SEQ ID NO:19]

Oligo #6 Length: 000036

25

GAGGTTGTTC GGGTCCAGCA AAGGGTTAGG TGGTCT [SEQ ID NO:20]

Oligo #7 Length: 000036

CACTTAAAGG TTCCACCTGC ACCTTTGCTG GACAGT [SEQ ID NO:21]

30

Oligo #8 Length: 000036

GAGGTTGTTA CTGTCCAGCA AAGGTGCAGG TGGAAC [SEQ ID NO:22]

Oligo #9 Length: 000027

AACAACCTCA ATGCTGAAGA CGTTGAT [SEQ ID NO:23]

35

Oligo #10 Length: 000018

86

ATCAACGTCT TCAGCATT [SEQ ID NO:24]

Oligo #11 Length: 000027

AACAACCTCA ATTCTGAAGA CATGGAT [SEQ ID NO:25]

5

Oligo #12 Length: 000018

ATCCATGTCT TCAGAATT [SEQ ID NO:26]

Oligo #13 Length: 000022

10 CATGGGAACC ATATGTCAGG AT [SEQ ID NO:27]

Oligo #14 Length: 000018

ATCCTGACAT ATGGTTCC [SEQ ID NO:28]

15 Oligo #15 Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:29]

Oligo #16 Length: 000024

AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:30]

20

Oligo #17 Length: 000020

AATTCGAACC ATATGTCAGA [SEQ ID NO:31]

Oligo #18 Length: 000020

25 AGCTTCTGAC ATATGGTTTCG [SEQ ID NO:32]

Oligo #19 Length: 000022

ATCGAACCAT ATGTCAGATG CA [SEQ ID NO:33]

30 Oligo #20 Length: 000018

TCTGACATAT GGTTCGAT [SEQ ID NO:34]

Oligo #21 Length: 000036

ATCCTGATGG AACGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:35]

35

Oligo #22 Length: 000027

AAGTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:36]

Oligo #23 Length: 000036

ATCCTGATGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:37]

5

Oligo #24 Length: 000027

AGTTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:38]

Oligo #25 Length: 000024

10 CTCGCATTCG TAAGGGCTGT CAAG [SEQ ID NO:39]

Oligo #26 Length: 000024

CCTTACGAAT GCGAGCAGGT TTGG [SEQ ID NO:40]

15 Oligo #27 Length: 000024

GAGAGCTTCG TAAGGGCTGT CAAG [SEQ ID NO:41]

Oligo #28 Length: 000024

20 CCTTACGAAG CTCTCCAGGT TTGG [SEQ ID NO:42]

Oligo #29 Length: 000015

CACTTAGAAA ATGCA [SEQ ID NO:43]

25 Oligo #30 Length: 000020

TTTCTAAGT GCTTGACAGC [SEQ ID NO:44]

Oligo #31 Length: 000015

AACTTAGAAA ATGCA [SEQ ID NO:45]

30

Oligo #32 Length: 000020

TTTCTAAGT TCTTGACAGC [SEQ ID NO:46]

Oligo #33 Length: 000048

35 GTGATTTGGA TGTCGAGAGG GTGCGCCGT GGCAGAGGGC AGACATGG
[SEQ ID NO:47]

- Oligo #45** Length: 000032
AATTCGGGA AAAACTGACG TTCTATCTGG TT [SEQ ID NO:59]
- 5 **Oligo #46** Length: 000037
CTCAAGGGAA ACCAGATAGA ACGTCAGTTT TTCCCGG [SEQ ID NO:60]
- Oligo #47** Length: 000032
ACCCTTGAGC ACGCGCAGGA ACAACAGTAA TA [SEQ ID NO:61]
- 10 **Oligo #48** Length: 000027
AGCTTATTAC TGTTGTTTCCT GCGCGTG [SEQ ID NO:62]
- Oligo #49** Length: 000032
15 ACCCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:63]
- Oligo #50** Length: 000027
AGCTTATTAC TGTTGTTTCCT GCGCTTG [SEQ ID NO:64]
- 20 **Oligo #51** Length: 000034
GCCGATACCGCGGCATACTCCCACCATTTCAGAGA [SEQ ID NO:155]
- Oligo #52** Length: 000033
25 GCCGATAAGATCTAAAACGGGTATGGAGAAACA [SEQ ID NO:156]
- Oligo #53**
ATAGTCTTCCCCAGATATCTAACGCTTGAG [SEQ ID NO:157]
- Oligo #54** Length: 24
30 CAATACCTGATGCGTTTTCTAAGT [SEQ ID NO:158]
- Oligo #55** Length: 33
GGTTTCGTTCCATCAGAATGTCCATGTCTTCAG [SEQ ID NO:159]
- 35 **Oligo #165** NCOECRV1.REQ Length: 000040.
CATGGCTAAC TGCTCTAACA TGATCGATGA AATTATAACA [SEQ ID
NO:162]
- 40 **Oligo #166** NCOECRV4.REQ Length: 000045

CTTTAAGTGT GTTATAATTT CATCGATCAT GTTAGAGCAG TTAGC [SEQ ID
NO:163]

5 Oligo #167 NCOECRV2.REQ Length: 000036

CACTTAAAGC AGCCACCTTT GCCTTTGCTG GACTTC [SEQ ID NO:164]

Oligo #168 NCOECRV5.REQ Length: 000036

10 GAGGTTGTTG AAGTCCAGCA AAGGCAAAGG TGGCTG [SEQ ID NO:165]

Oligo #169 2D5M6SUP.REQ Length: 000027

15 AACAACTCA ATGACGAAGA CATGTCT [SEQ ID NO:166]

Oligo #170 2D5M6SLO.REQ Length: 000018

AGACATGTCT TCGTCATT [SEQ ID NO:167]

20 Oligo #15(A) Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:168]

Oligo #16(A) Length: 000024

25 AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:169]

Oligo #B1 19ALA1.REQ Length: 000040

CATGGCAAAC TGCTCTATAG CTATCGATGA AATTATACAT [SEQ ID NO:170]

30 Oligo #B2 19ALA4.REQ Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATAGC TATAGAGCAG TTTGC [SEQ ID
NO:171]

35 Oligo #B3 19ILE1.REQ Length: 000040

CATGGCAAAC TGCTCTATAA TCATCGATGA AATTATACAT [SEQ ID NO:172]

40 Oligo #B4 19ILE4.REQ Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATGAT TATAGAGCAG TTTGC [SEQ ID
NO:173]

45 Oligo #B5 49ASP1.REQ Length: 000036

ATCCTGGACG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:174]

Oligo #B6 49ASP4.REQ Length: 000027

50 AGTTCGAAGG TTTCGTTCGT CCAGGAT [SEQ ID NO:175]

Oligo #B7 49ILE1.REQ Length: 000036

ATCCTGATCG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:176]

5 Oligo #B8 49ILE4.REQ Length: 000027
AGTTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:177]

Oligo #B9 49LEU1.REQ Length: 000036

10 ATCCTGCTGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:178]

Oligo #B10 49LEU4.REQ Length: 000027
AGTTCGAAGG TTTCGTTCCA GCAGGAT [SEQ ID NO:179]

15 Oligo #B11 42S45V3.REQ Length: 000027
AACAACCTCA ATTCTGAAGA CGTTGAT [SEQ ID NO:180]

20 Oligo #B12 42S45V6.REQ Length: 000018
ATCAACGTCT TCAGAATT [SEQ ID NO:181]

Oligo #B13 18I23A5H.REQ Length: 000051

25 CGCGCCATGG CTAAGTCTC TATAATGATC GATGAAGCAA TACATCACTTA
[SEQ ID NO:182]

Oligo #B14 2341HIN3.REQ Length: 000018

30 CGCGTCGATA AGCTTATT [SEQ ID NO:183]

Oligo #B15 2341NCO.REQ Length: 000018

35 GGAGATATAT CCATGGCT [SEQ ID NO:184]

Oligo #B16 2A5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCAGC ATTGAGGTTG TT [SEQ ID
40 NO:185]

Oligo #B17 2A5V6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGAAA CGTCTTCAGC ATTGAGGTTG TT [SEQ ID
45 NO:186]

Oligo #B18 2D5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCGTC ATTGAGGTTG TT [SEQ ID
50 NO:187]

Oligo #B19 2D5V6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGAAA CGTCTTCGTC ATTGAGGTTG TT [SEQ ID
55 NO:188]

Oligo #B20 2S5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCAGA ATTGAGGTTG TT [SEQ ID
60 NO:189]

Oligo #B21 2S5V6S0D.REQ Length: 000042
 TCGGTCCATC AGAATAGAAA CGTCTTCAGA ATTGAGGTTG TT [SEQ ID
 NO:190]

5 Oligo #B22 100ARG3.REQ Length: 000048
 CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA TCATCCGT [SEQ
 ID NO:191]

10 Oligo #B23 100ARG8.REQ Length: 000026
 AATTCTTGCC AGTCACCTGC ACGGAT [SEQ ID NO:192]

15 Oligo #B24 101MET4.REQ Length: 000016
 ATGGGTGACT GGCAAG [SEQ ID NO:193].

20 Oligo #B25 10R01M8.REQ Length: 000026
 AATTCTTGCC AGTCACCCAT ACGGAT [SEQ ID NO:194]

 Oligo #B26 23ALA1.REQ Length: 000040
 CATGGCTAAC TGCTCTATTA TGATCGATGA AGCAATACAT [SEQ ID NO:195]

25 Oligo #B27 23ALA4.REQ Length: 000045
 CTTTAAGTGA TGTATTGCTT CATCGATCAT AATAGAGCAG TTAGC [SEQ ID
 NO:196]

30 Oligo #B28 29V2R4S2.REQ Length: 000036
 CACTTAAAGG TACCACCTCG CCCTTCCCTG GACCCG [SEQ ID NO:197]

35 Oligo #B29 29V2R4S5.REQ Length: 000036
 GAGGTTGTTC GGGTCCAGGG AAGGGCGAGG TGGTAC [SEQ ID NO:198]

40 Oligo #B30 34SER2.REQ Length: 000036
 CACTTAAAGA GACCACCTGC ACCTTCCCTG GACCCG [SEQ ID NO:199]

45 Oligo #B31 34SER5.REQ Length: 000036
 GAGGTTGTTC GGGTCCAGGG AAGGTGCAGG TGGTCT [SEQ ID NO:200]

 Oligo #B32 42D45M3.REQ Length: 000027
 AACAACCTCA ATGACGAAGA CATGGAT [SEQ ID NO:201]

50 Oligo #B33 42D45M6.REQ Length: 000018
 ATCCATGTCT TCGTCATT [SEQ ID NO:202]

55 Oligo #B34 42D45V3.REQ Length: 000027
 AACAACCTCA ATGACGAAGA CGTCGAT [SEQ ID NO:203]

60 Oligo #B35 42D45V6.REQ Length: 000018

ATCGACGTCT TCGTCATT [SEQ ID NO:204]

Oligo #B36 42D5M6S3.REQ Length: 000027

5 AACAACTCA ATGACGAAGA CATGTCT [SEQ ID NO:205]

Oligo #B37 42D5M6S6.REQ Length: 000018

AGACATGTCT TCGTCATT [SEQ ID NO:206]

10 Oligo #B38 42D5V6S3.REQ Length: 000027

AACAACCTCA ATGACGAAGA CGTCTCT [SEQ ID NO:207]

15 Oligo #B39 42D5V6S6.REQ Length: 000018

AGAGACGTCT TCGTCATT [SEQ ID NO:208]

Oligo #B40 50ASP1.REQ Length: 000036

20 ATCCTGATGG ACCGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:209]

Oligo #B41 50ASP4.REQ Length: 000027

25 AAGTCGAAGG TTTCGGTCCA TCAGGAT [SEQ ID NO:210]

Oligo #B42 50D56S1.REQ Length: 000036

ATCCTGATGG ACCGAAACCT TCGACTTAGC AACCTG [SEQ ID NO:211]

30 Oligo #B43 56SER5.REQ Length: 000024

CCTTACGAAG CTCTCCAGGT TGCT [SEQ ID NO:212]

35 Oligo #B44 82TRP2.REQ Length: 000018

CGTAATCTCT GGCCATGT [SEQ ID NO:213]

Oligo #B45 82TRP6.REQ Length: 000018

40 CCAGAGATTA CGAAGAAT [SEQ ID NO:214]

Oligo #B46 9E12Q6W1.REQ Length: 000032
 AATTCCGGGA AAAACTGCAA TTCTATCTGT GG [SEQ ID NO:215]

5 Oligo #B47 9E12Q6W3.REQ Length: 000037
 CTCAAGGGTC CACAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:216]

10 Oligo #B48 9E12Q6V1.REQ Length: 000032
 AATTCCGGGA AAAACTGCAA TTCTATCTGG TT [SEQ ID NO:217]

 Oligo #B49 9E12Q6V3.REQ Length: 000037
 15 CTCAAGGGTA ACCAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:218]

 Oligo #B50 S09E16V1.REQ Length: 000023
 AATTCCGGGA AAAACTGACG TTC [SEQ ID NO:219]

20 Oligo #B51 S09E16V3.REQ Length: 000028
 AACCAGATAG AACGTCAGTT TTTCCCGG [SEQ ID NO:220]

25 Oligo #B52 S116VD31.REQ Length: 000023
 TATCTGGTTA CCCTTGAGTA ATA [SEQ ID NO:221]

 Oligo #B53 SECR1D33.REQ Length: 000018
 30 AGCTTATTAC TTCAAGGGT [SEQ ID NO:222]

 Oligo #B54 S9E2Q6V1.REQ Length: 000023
 35 AATTCCGGGA AAAACTGCAA TTC [SEQ ID NO:223]

 Oligo #B55 S9E2Q6V3.REQ Length: 000028
 AACCAGATAG AATTGCAGTT TTTCCCGG [SEQ ID NO:224]

40 Oligo #B56 Ent338.Lo Length: 61
 CGATCATTAT AGAGCAGTTA GCCTTGTCAT CGTCGTCCTT GTAATCAGTT
 TCTGGATATG C [SEQ ID NO:225]

45 Oligo #B57 Ent338.UP Length: 63
 CATGGCATAT CCAGAACTG ATTACAAGGA CGACGATGAC AAGGCTAACT
 GCTCTATAAT GAT SEQ ID NO:226]

50 09L2Q6S1.REQ Length: 000032
 AATTCCGGCT TAAACTGCAA TTCTATCTGT CT [SEQ ID NO:227]

55 09L2Q6S3.REQ Length: 000037
 CTCAAGGGTA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ ID NO:228]
 117S2.REQ Length: 000032

60 TCTCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:229]

95

19I0L3A1.REQ Length: 000040
 CATGGCAAAC TGCTCTATAA TACTCGATGA AGCAATACAT [SEQ ID NO:230]

5
 19I0L3A4.REQ Length: 000045
 CTTTAAGTGA TGTATTGCTT CATCGAGTAT TATAGAGCAG TTTGC [SEQ. ID
 NO.:231]

10
 20P23A1.REQ Length: 000040
 CATGGCAAAC TGCTCTATAA TGCCAGATGA AGCAATACAT [SEQ. ID
 NO.:232]

15
 20P23A4.REQ Length: 000045
 CTTTAAGTGA TGTATTGCTT CATCTGGCAT TATAGAGCAG TTTGC [SEQ. ID
 NO.:233]

20
 23L1.REQ Length: 000040
 CATGGCaAAC TGCTCTATAA TGATCGATGA AactgATACAT [SEQ. ID
 NO.:234]

25
 23L4.REQ Length: 000045
 CTTTAAGTGA TGTATcagTT CATCGATCAT TATAGAGCAG TTtGC [SEQ. ID
 NO.:235]

30
 29I4S7S2.REQ Length: 000036
 CACTTAAAGA TACCACCTAA CCCTAGCCTG GACAGT [SEQ. ID NO.:236]

35
 29I4S7S5.REQ Length: 000036
 GAGGTTAGCA CTGTCCAGGC TAGGGTTAGG TGGTAT [SEQ. ID NO.:237]

40
 38A5V6S3.REQ Length: 000027
 GCTAACCTCA ATTCCGAAGA CGTCTCT [SEQ. ID NO.:238]

45
 38A5V6S6.REQ Length: 000018
 AGAGACGTCT TCGGAATT [SEQ. ID NO.:239]

50
 50D51S1.REQ Length: 000036
 ATCCTGATGG ACTCCAACCT TCGAACTCCA AACCTG [SEQ. ID NO.:240]

50
 50D51S4.REQ Length: 000027
 AGTTCGAAGG TTGGAGTCCA TCAGGAT [SEQ. ID NO.:241]

55
 5VYWPTT3.REQ Length: 000048
 GTTCCCTATT GGACGGCCCC TCCCTCTCGA ACACCAATCA CGATCAAG [SEQ. ID
 NO.:242]

60
 5VYWPTT7.REQ Length: 000048

CGTGATTGGT GTTCGAGAGG GAGGGGCCGT CCAATAGGGA ACACATGG [SEQ. ID NO.:243]

5 62P3H5S2.REQ Length: 000024
CTCGCATTC CACATGCTTC TAAG [SEQ. ID NO.:244]

62P63H2.REQ Length: 000024

10 CTCGCATTCC CACATGCTGT CAAG [SEQ. ID NO.:245]

62P63H5.REQ Length: 000024

15 ATGTGGGAAT GCGAGCAGGT TTGG [SEQ. ID NO.:246]

65S67Q6.REQ Length: 000020

TTTTCTAATT GCTTAGAAGC [SEQ. ID NO.:247]

20 67Q3.REQ Length: 000015

CAATTAGAAA ATGCA [SEQ. ID NO.:248]

67Q6.REQ Length: 00002]

25 TTTTCTAATT GCTTGACAGC [SEQ. ID NO.:249]

76P1.REQ Length: 000021

30 TCAGGTATTG AGCCAATTCT T [SEQ. ID NO.:250]

76P5.REQ Length: 000019

TGGCTCAATA CCTGATGCA [SEQ. ID NO.:251]

35 79S2.REQ Length: 000018

TCTAATCTCC AACCATGT [SEQ. ID NO.:252]

40 79S6.REQ Length: 000018

TTGGAGATTA GAAAGAAT [SEQ. ID NO.:253]

9L2Q67S3.REQ Length: 000037

45 CTCAAGAGAA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ. ID NO.:254]

9LQS1181.REQ Length: 000043

50 AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID NO.:256]

9LQS1183.REQ Length: 000043

55 AGCTTATTAA AGGGTAGACA GATAGAATTG CAGTTTAAGC CGG [SEQ. ID NO.:257]

S9L2Q6S1.REQ Length: 000043

60 AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID

		Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
		15					20					25		
5	Lys	Arg	Pro	Pro	Ala	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn
			30					35					40	
	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Asn	Asn	Leu	Arg	Arg	Pro
			45					50					55	
10	Leu	Glu	Ala	Phe	Asn	Arg	Ala	Val	Lys	Ser	Leu	Gln	Asn	Ala
			60					65					70	
	Ala	Ile	Glu	Ser	Ile	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro
15			75					80					85	
	Ala	Thr	Ala	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp
			90					95					100	
20	Asp	Trp	Asn	Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys
			105					110					115	
	Leu	Glu	Asn	Ala	Gln	Ala	Gln	Gln	[SEQ ID NO:66]					
			120					125						
25														
	PEPTIDE #3; pMON13345 (Example 9); (15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S and 45M);													
30														
		Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
		15					20					25		
	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn
35			30					35					40	
	Glu	Asp	Met	Asp	Ile	Leu	Met	Glu	Asn	Asn	Leu	Arg	Arg	Pro
			45					50					55	
40	Leu	Glu	Ala	Phe	Asn	Arg	Ala	Val	Lys	Ser	Leu	Gln	Asn	Ala
			60					65					70	
	Ala	Ile	Glu	Ser	Ile	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro
			75					80					85	
45	Ala	Thr	Ala	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp
			90					95					100	
	Asp	Trp	Asn	Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys
50			105					110					115	
	Leu	Glu	Asn	Ala	Gln	Ala	Gln	Gln	[SEQ ID NO:67]					
			120					125						
55														
	PEPTIDE #4; pMON13346 (Example 10); (15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S and 45M);													
60														
		Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
		15					20					25		

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
30 35 40

5 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45 50 55

10 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

15 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105 110 115

20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]
120 125

25 **PEPTIDE #5**; pMON13347 (Example 12); (15-125)hIL-3 (51R, 55L, 59L,
62V,
67N and 69E);

30 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 35 40

35 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

40 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

45 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105 110 115

50 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]
120 125

55 **PEPTIDE #6**; pMON13348 (Example 13); (15-125)hIL-3 (51R, 55L, 60S,
62V,
67N and 69E);

60 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

100

		30		35		40	
	Glu Asp	Gln Asp Ile Leu Met	Glu Arg Asn Leu Arg	Leu Pro Asn			
		45	50	55			
5							
	Leu Glu	Ser Phe Val Arg Ala	Val Lys Asn Leu Glu	Asn Ala Ser			
		60	65	70			
10	Ala Ile	Glu Ser Ile Leu Lys	Asn Leu Leu Pro Cys	Leu Pro Leu			
		75	80	85			
	Ala Thr	Ala Ala Pro Thr Arg	His Pro Ile His Ile	Lys Asp Gly			
		90	95	100			
15	Asp Trp	Asn Glu Phe Arg Arg	Lys Leu Thr Phe Tyr	Leu Lys Thr			
		105	110	115			
20	Leu Glu	Asn Ala Gln Ala Gln	Gln [SEQ ID NO:70]				
		120	125				
	PEPTIDE #7; pMON13349 (Example 14); (15-125)hIL-3 (51R, 55T, 59L, 62V,						
25		67H and 69E);					
		Asn Cys Ser Asn Met	Ile Asp Glu Ile Ile Thr His Leu				
		15	20	25			
30	Lys Gln	Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn	Leu Asn Gly			
		30	35	40			
	Glu Asp	Gln Asp Ile Leu Met	Glu Arg Asn Leu Arg	Thr Pro Asn			
		45	50	55			
35	Leu Leu	Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser			
		60	65	70			
	Ala Ile	Glu Ser Ile Leu Lys	Asn Leu Leu Pro Cys	Leu Pro Leu			
		75	80	85			
40	Ala Thr	Ala Ala Pro Thr Arg	His Pro Ile His Ile	Lys Asp Gly			
		90	95	100			
45	Asp Trp	Asn Glu Phe Arg Arg	Lys Leu Thr Phe Tyr	Leu Lys Thr			
		105	110	115			
	Leu Glu	Asn Ala Gln Ala Gln	Gln [SEQ ID NO:71]				
		120	125				
50							
	PEPTIDE #8; pMON13350 (Example 16); (15-125)hIL-3 (73G, 76A, 79R, 82Q, 87S,						
55		93S, 98I, 101A and 105Q);					
		Asn Cys Ser Asn Met	Ile Asp Glu Ile Ile Thr His Leu				
		15	20	25			
60	Lys Gln	Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn	Leu Asn Gly			

	30		35		40
	Glu Asp Gln Asp Ile Leu Met	Glu Asn Asn Leu Arg	Arg Pro Asn		
	45	50	55		
5	Leu Glu Ala Phe Asn Arg Ala	Val Lys Ser Leu Gln Asn Ala Ser			
	60	65	70		
10	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys Leu Pro Ser			
	75	80	85		
	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile Lys Ala Gly			
	90	95	100		
15	Asp Trp Gln Glu Phe Arg Arg	Lys Leu Thr Phe Tyr Leu Lys Thr			
	105	110	115		
	Leu Glu Asn Ala Gln Ala Gln	Gln [SEQ ID NO:72]			
	120	125			
20	PEPTIDE #9; pMON13355 (Example 17); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A and 105Q);				
25	Asn Cys Ser Asn Met	Ile Asp Glu Ile Ile Thr His Leu			
	15	20	25		
	Lys Gln Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn Leu Asn Gly			
	30	35	40		
30	Glu Asp Gln Asp Ile Leu Met	Glu Asn Asn Leu Arg Arg Pro Asn			
	45	50	55		
35	Leu Glu Ala Phe Asn Arg Ala	Val Lys Ser Leu Gln Asn Ala Ser			
	60	65	70		
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Val Pro Cys Leu Pro Ser			
	75	80	85		
40	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Thr Ile Lys Ala Gly			
	90	95	100		
	Asp Trp Gln Glu Phe Arg Arg	Lys Leu Thr Phe Tyr Leu Lys Thr			
	105	110	115		
45	Leu Glu Asn Ala Gln Ala Gln	Gln [SEQ ID NO:73]			
	120	125			
50	PEPTIDE #10; pMON13352 (Example 19); (15-125)hIL-3 (109E, 116V, 120Q and 123E);				
55	Asn Cys Ser Asn Met	Ile Asp Glu Ile Ile Thr His Leu			
	15	20	25		
	Lys Gln Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn Leu Asn Gly			
	30	35	40		
60	Glu Asp Gln Asp Ile Leu Met	Glu Asn Asn Leu Arg Arg Pro Asn			
	45	50	55		

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Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]
120 125

PEPTIDE #11; pMON13354 (Example 20); (15-125)hIL-3 (109E, 116V,
20 117S, 120H
and 123E);

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

25 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 35 40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
30 45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

40 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
105 110 115

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:75]
45 120 125

PEPTIDE #12; pMON13360 (Example 21); (15-125)hIL-3 (73G, 76A,
79R, 82Q,
50 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

55 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 35 40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45 50 55

60 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

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	60	65	70
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser
	75	80	85
5	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly
	90	95	100
10	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr
	105	110	115
	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ. NO:76]	
	120	125	
15	PEPTIDE #13: pMON13361 (Example 22); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);		
20	Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu		
	15	20	25
25	Lys Gln Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn Leu Asn Gly	
	30	35	40
	Glu Asp Gln Asp Ile Leu Met	Glu Asn Asn Leu Arg Arg Pro Asn	
	45	50	55
30	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser		
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Val Pro Cys Leu Pro Ser	
	75	80	85
35	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Thr Ile Lys Ala Gly	
	90	95	100
40	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr Leu Val Thr	
	105	110	115
	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ ID NO:77]	
	120	125	
45	PEPTIDE #14: pMON13362 (Example 23); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E);		
50	Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu		
	15	20	25
55	Lys Gln Pro Pro Leu Pro Leu	Leu Asp Phe Asn Asn Leu Asn Gly	
	30	35	40
	Glu Asp Gln Asp Ile Leu Met	Glu Asn Asn Leu Arg Arg Pro Asn	
	45	50	55
60	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser		

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	60		65		70
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Val Pro Cys	Leu Pro Ser		
	75	80	85		
5	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Thr Ile	Lys Ala Gly		
	90	95	100		
10	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Ser		
	105	110	115		
	Leu Glu His Ala Gln Glu Gln	[SEQ ID NO:78]			
	120	125			
15	PEPTIDE #15; pMON13363 (Example 24); (15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E);				
	Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu		
20	15	20	25		
	Lys Arg Pro Pro Ala Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ala		
	30	35	40		
25	Glu Asp Val Asp Ile Leu Met	Glu Arg Asn Leu Arg	Leu Pro Asn		
	45	50	55		
	Leu Glu Ser Phe Val Arg Ala	Val Lys Asn Leu Glu	Asn Ala Ser		
	60	65	70		
30	Ala Ile Glu Ser Ile Leu Lys	Asn Leu Leu Pro Cys	Leu Pro Leu		
	75	80	85		
	Ala Thr Ala Ala Pro Thr Arg	His Pro Ile His Ile	Lys Asp Gly		
35	90	95	100		
	Asp Trp Asn Glu Phe Arg Arg	Lys Leu Thr Phe Tyr	Leu Lys Thr		
	105	110	115		
40	Leu Glu Asn Ala Gln Ala Gln	Gln [SEQ ID NO:79]			
	120	125			
45	PEPTIDE #16; pMON13364 (Example 25); (15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E);				
	Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu		
	15	20	25		
50	Lys Arg Pro Pro Asn Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ser		
	30	35	40		
55	Glu Asp Met Asp Ile Leu Met	Glu Arg Asn Leu Arg	Thr Pro Asn		
	45	50	55		
	Leu Leu Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser		
	60	65	70		
60	Ala Ile Glu Ser Ile Leu Lys	Asn Leu Leu Pro Cys	Leu Pro Leu		
	75	80	85		

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	90	95	100
	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr		
	105	110	115
5	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82]		
	120	125	
10	PEPTIDE #19; pMON13299 (Example 28); Met-Ala-(15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);		
15	Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu		
	15	20	25
	Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly		
	30	35	40
20	Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn		
	45	50	55
	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser		
25	60	65	70
	Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser		
	75	80	85
30	Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly		
	90	95	100
	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr		
	105	110	115
35	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83]		
	120	125	
40	PEPTIDE #20; pMON13300 (Example 29); Met-Ala-(15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E);		
	Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu		
	15	20	25
45	Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly		
	30	35	40
	Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn		
	45	50	55
50	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser		
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser		
	75	80	85
55	Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly		
	90	95	100
60	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser		

60

60 **PEPTIDE #25;** pMON13288 (Example 34); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and

123E);

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25
Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40
10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70
15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]
120 125

30 **PEPTIDE #26;** pMON13289 (Example 35); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25
Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
30 35 40
40 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55
Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70
45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]
120 125

60 **PEPTIDE #27;** pMON13290 (Example 36); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

5 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

10 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:91]
120 125

PEPTIDE #28; pMON13292 (Example 37); Met-Ala-(15-125)hIL-3 (18I,
25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G,
30 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and
123E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

35 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
30 35 40

40 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]
120 125

55

PEPTIDE #29; pMON13294 (Example 38); Met-Ala-(15-125)hIL-3 (18I,
25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G,
60 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H
and 123E);

	15	20	25
	Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser		
	30	35	40
5	Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn		
	45	50	55
10	Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser		
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser		
	75	80	85
15	Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly		
	90	95	100
20	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr		
	105	110	115
	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:95]		
	120	125	
25	PEPTIDE #32; pMON13313 (Example 41); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E);		
30	Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu		
	15	20	25
35	Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala		
	30	35	40
	Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn		
	45	50	55
40	Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser		
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser		
	75	80	85
45	Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly		
	90	95	100
50	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser		
	105	110	115
	Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:96]		
	120	125	
55	PEPTIDE #A3; pMON13285 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 50D);		
60	Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu		
	15	20	25

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Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
30 35 40

5 Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

10 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105 110 115

20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:259]
120 125

PEPTIDE #A4; pMON13286 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S);

25 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
30 35 40

Glu Asp **Met Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45 50 55

35 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

40 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105 110 115

45 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:260]
120 125

50 **PEPTIDE #A5; pMON13325 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 116W);**

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

55 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
30 35 40

Glu Asp **Met Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45 50 55

60

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

10 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:261]
120 125

15

PEPTIDE #A6; pMON13326 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 50D, 116W);

20 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
30 35 40

25 Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
45 50 55

30 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

35 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
105 110 115

40 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:262]
120 125

45

PEPTIDE #A7; pMON13330 Met-Ala-IL-3; (42D, 45M, 46S, 50D, 95R, 98I, 100R, 116W);

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25

50 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
30 35 40

Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70

60 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85

115

Ala Thr Ala Ala Pro Thr Arg **Arg** Pro Ile **Ile** Ile **Arg** Asp Gly
 90 95 100

5 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:263]
 120 125

10

PEPTIDE #A8; pMON13329 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S,
 98I, 100R, 116W);

15 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25

20 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40

Glu Asp **Met Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55

25 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

30 Ala Thr Ala Ala Pro Thr Arg His Pro Ile **Ile** Ile **Arg** Asp Gly
 90 95 100

35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:406]
 120 125

40

PEPTIDE #B1 Met-Ala-(15-125)hIL-3 pMON13406

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
 15 20 25

45 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

116

105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 264]
120 125

5

PEPTIDE # B2 Met-Ala-(15-125)hIL-3 pMON13414

10 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40

15 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 265]
120 125

35

PEPTIDE #B3 Met-Ala-(15-125)hIL-3 pMON13407

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 266]
120 125

60

PEPTIDE #B4 Met-Ala-(15-125)hIL-3 pMON13405

117

	Met	Ala	Asn	Cys	Ser	Ile	Ala	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
5	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
10	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		
	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
15			75					80					85		
	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
20	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln [SEQ ID NO.: 267]							
			120				125								
25															
	PEPTIDE #B5 Met-Ala-(15-125)hIL-3 pMON13415														
	Met	Ala	Asn	Cys	Ser	Ile	Ile	Ile	Asp	Glu	Ile	Ile	His	His	Leu
30			15					20					25		
	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
35	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		
40	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
45			90					95					100		
	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
50	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln [SEQ ID NO.: 268]							
			120				125								
	PEPTIDE #B6 Met-Ala-(15-125)hIL-3 pMON13408														
55	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
60			30					35					40		

118

	Glu Asp Met Asp Ile Leu Ile	Glu Arg Asn Leu Arg	Thr Pro Asn
	45	50	55
5	Leu Leu Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser
	75	80	85
10	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly
	90	95	100
	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr
	105	110	115
15	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 269]	
	120	125	
20	PEPTIDE #B7 Met-Ala-(15-125)hIL-3 pMON13409		
	Met Ala Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu
	15	20	25
25	Lys Arg Pro Pro Asn Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ser
	30	35	40
	Glu Asp Met Asp Ile Leu Leu	Glu Arg Asn Leu Arg	Thr Pro Asn
	45	50	55
30	Leu Leu Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser
	60	65	70
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser
	75	80	85
35	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly
	90	95	100
40	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr
	105	110	115
	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 270]	
	120	125	
45	PEPTIDE #B8 Met-Ala-(15-125)hIL-3 pMON13410		
	Met Ala Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu
	15	20	25
50	Lys Arg Pro Pro Asn Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ser
	30	35	40
	Glu Asp Met Asp Ile Leu Asp	Glu Arg Asn Leu Arg	Thr Pro Asn
	45	50	55
	Leu Leu Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser
	60	65	70
60	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser

119

	75		80		85
	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
5	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr	
	105		110	115	
10	Leu Glu	Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 271]		
	120		125		
PEPTIDE #B9 Met-Ala-(15-125)hIL-3 pMON13422					
15	Met Ala	Asn Cys Ser Ile Ala	Ile Asp Glu Ile Ile	His His Leu	
	15		20	25	
	Lys Arg	Pro Pro Asn Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ser	
	30		35	40	
20	Glu Asp	Val Asp Ile Leu Ile	Glu Arg Asn Leu Arg	Thr Pro Asn	
	45		50	55	
	Leu Leu	Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser	
25	60		65	70	
	Gly Ile	Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser	
	75		80	85	
30	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr	
	105		110	115	
35	Leu Glu	Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 272]		
	120		125		
40 PEPTIDE #B10 Met-Ala-(15-125)hIL-3 pMON13423					
	Met Ala	Asn Cys Ser Ile Ile	Ile Asp Glu Ile Ile	His His Leu	
	15		20	25	
45	Lys Arg	Pro Pro Asn Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ser	
	30		35	40	
	Glu Asp	Val Asp Ile Leu Ile	Glu Arg Asn Leu Arg	Thr Pro Asn	
	45		50	55	
50	Leu Leu	Ala Phe Val Arg Ala	Val Lys His Leu Glu	Asn Ala Ser	
	60		65	70	
	Gly Ile	Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser	
55	75		80	85	
	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
60	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr	
	105		110	115	

120

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 273]
120 125

5

PEPTIDE #B11 Met-Ala-(15-125)hIL-3 pMON13424

10 Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
15 15 20 25
Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40
15 Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70
20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 274]
120 125

35 PEPTIDE #B12 Met-Ala-(15-125)hIL-3 pMON13425

Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu
15 20 25
40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40
Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
55 105 110 115
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 275]
120 125

60

PEPTIDE #B13 Met-Ala-(15-125)hIL-3 pMON13426

121

	Met	Ala	Asn	Cys	Ser	Ile	Ala	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
5	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
10	Glu	Asp	Val	Asp	Ile	Leu	Asp	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		
15	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
20	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
25	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 276]						
			120					125							
30	PEPTIDE #B14 Met-Ala-(15-125)hIL-3 pMON13429														
	Met	Ala	Asn	Cys	Ser	Ile	Ile	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
35	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
	Glu	Asp	Val	Asp	Ile	Leu	Asp	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
40	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		
	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
45	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
50	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 277]						
			120					125							
55	PEPTIDE #B15 Met-Ala-(15-125)hIL-3 pMON13368														
	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ala	Ile	His	His	Leu
			15					20					25		
60	Lys	Val	Pro	Pro	Ala	Pro	Leu	Leu	Asp	Ser	Asn	Asn	Leu	Asn	Ser

123

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 280]
120 125

15 PEPTIDE #B18 Met-Ala-(15-125)hIL-3 pMON13366

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asn
30 35 40

Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 281]
120 125

40

PEPTIDE #B19 Met-Ala-(15-125)hIL-3 pMON13367

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

45 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

50 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

124

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    Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
      105      110      115
5    Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 282]
      120      125

PEPTIDE #B20 Met-Ala-(15-125)hIL-3 pMON13369
10   Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
      15      20      25

    Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
      30      35      40
15   Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
      45      50      55

    Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
      60      65      70
20   Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
      75      80      85

    Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
      90      95     100
25   Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
      105      110      115
30   Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 283]
      120      125

35   PEPTIDE #B21 Met-Ala-(15-125)hIL-3 pMON13370

    Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
      15      20      25

    Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
      30      35      40
40   Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
      45      50      55

    Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
      60      65      70
45   Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
      75      80      85

    Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
      90      95     100
50   Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
      105      110      115

    Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 284]
      120      125
60

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126

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu [SEQ ID NO.: 287]
119

20 PEPTIDE #B25 Met-Asp-(15-119)hIL-3 pMON13376

Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

25 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

30 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
105 110 115

Leu Glu [SEQ ID NO.: 288]
119

45 PEPTIDE #B26 Met-Ala-(15-125)hIL-3 pMON13377

50 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

55 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
45 50 55

60 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

127

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 289]
120 125

15 PEPTIDE #B27 Met-Asp-(15-119)hIL-3 pMON13378

Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu [SEQ ID NO.: 290]
119

40 PEPTIDE #B28 Met-Ala-(15-125)hIL-3 pMON13379

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

45 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

50 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr

128

105

110

115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 291]

120

125

5

PEPTIDE #B29 Met-Ala-(15-125)hIL-3 pMON13385

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

15 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 292]
120 125

35 PEPTIDE #B30 Met-Ala-(15-125)hIL-3 pMON13381

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

45 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Trp Pro Cys Leu Pro Ser
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 293]
120 125

60

PEPTIDE #B31 Met-Ala-(15-125)hIL-3 pMON13383

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25

5 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40

Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55

10 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
 105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 294]
 120 125

PEPTIDE #B32 Met-Ala-(15-125)hIL-3 pMON13384

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

35 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
 105 110 115

50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 295]
 120 125

55 PEPTIDE #B33 Met-Ala-(15-125)hIL-3 pMON13388

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

60 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

	Glu	Asp	Val	Asp	Ile	Leu	Met	Asp	Arg	Asn	Leu	Arg	Leu	Ser	Asn
			45					50						55	
5	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65					70		
	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
10	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
15	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 296]						
			120					125							
20	PEPTIDE #B34 Met-Ala-(15-125)hIL-3 pMON13389														
	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
25	Lys	Arg	Pro	Pro	Ala	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Asp
			30					35					40		
	Glu	Asp	Met	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn
			45					50					55		
30	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65					70		
35	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
40	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
45	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 297]						
			120					125							
	PEPTIDE #B35 Met-Ala-(15-125)hIL-3 pMON13391														
50	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
	Lys	Arg	Pro	Pro	Ala	Pro	Ser	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ala
			30					35					40		
55	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn
			45					50					55		
60	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65					70		

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Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 298]
120 125

PEPTIDE #B36 Met-Ala-(15-125)hIL-3 pMON13392

15 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 299]
120 125

40 PEPTIDE #B37 Met-Ala-(15-125)hIL-3 pMON13393

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

45 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 300]
120 125

5

PEPTIDE #B38 Met-Ala-(15-125)hIL-3 pMON13394

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

15 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 301]
120 125

35

PEPTIDE #B39 Met-Ala-(15-125)hIL-3 pMON13395

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

40 Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 302]
120 125

60

PEPTIDE #B40 Met-Ala-(15-125)hIL-3 pMON13396

134

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 305]
120 125

20 PEPTIDE #B43 Met-Ala-(15-125)hIL-3 pMON13399

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

25 Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

30 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

35 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 306]
120 125

45

PEPTIDE #B44 Met-Ala-(15-119)hIL-3 pMON13404

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

50 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

135

	75		80		85
	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
5	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Gln Phe Tyr	Leu Val Thr	
	105		110	115	
10	Leu Glu	[SEQ ID NO.: 307]			
	119				

PEPTIDE #B45 Met-Ala-(15-125)hIL-3 pMON13387

15	Met Ala	Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu	
	15		20	25	
	Lys Arg	Pro Pro Ala Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Ala	
	30		35	40	
20	Glu Asp	Val Asp Ile Leu Met	Asp Arg Asn Leu Arg	Leu Pro Asn	
	45		50	55	
25	Leu Glu	Ser Phe Val Arg Ala	Val Lys Asn Leu Glu	Asn Ala Ser	
	60		65	70	
	Gly Ile	Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser	
	75		80	85	
30	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr	
	105		110	115	
35	Leu Glu	Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 308]		
	120		125		

40 PEPTIDE #B46 Met-Ala-(15-125)hIL-3 pMON13416

	Met Ala	Asn Cys Ser Ile Met	Ile Asp Glu Ile Ile	His His Leu	
	15		20	25	
45	Lys Arg	Pro Pro Ala Pro Leu	Leu Asp Pro Asn Asn	Leu Asn Asp	
	30		35	40	
	Glu Asp	Val Ser Ile Leu Met	Glu Arg Asn Leu Arg	Leu Pro Asn	
	45		50	55	
50	Leu Glu	Ser Phe Val Arg Ala	Val Lys Asn Leu Glu	Asn Ala Ser	
	60		65	70	
	Gly Ile	Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys	Leu Pro Ser	
	75		80	85	
55	Ala Thr	Ala Ala Pro Ser Arg	His Pro Ile Ile Ile	Lys Ala Gly	
	90		95	100	
60	Asp Trp	Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr	Leu Val Thr	
	105		110	115	

136

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 309]
120 125

5

PEPTIDE #B47 Met-Ala-(15-125)hIL-3 pMON13417

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25
10 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40
15 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70
20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 310]
120 125

PEPTIDE #B48 Met-Ala-(15-125)hIL-3 pMON13420

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25
Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40
40 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn
45 50 55
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85
50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 311]
120 125

60 PEPTIDE #B49 Met-Ala-(15-125)hIL-3 pMON13421

137

	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ala	Ile	His	His	Leu
			15					20					25		
5	Lys	Arg	Pro	Pro	Ala	Pro	Ser	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Asp
			30					35					40		
	Glu	Asp	Met	Ser	Ile	Leu	Met	Asp	Arg	Asn	Leu	Arg	Leu	Ser	Asn
			45					50					55		
10	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65					70		
	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
15	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
20	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.:331]						
			120					125							
25	PEPTIDE #B50 Met-Ala-(15-125)hIL-3 pMON13432														
	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ala	Ile	His	His	Leu
			15					20					25		
30	Lys	Arg	Pro	Pro	Ala	Pro	Ser	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Asp
			30					35					40		
	Glu	Asp	Met	Ser	Ile	Leu	Met	Asp	Arg	Asn	Leu	Arg	Leu	Pro	Asn
35			45					50					55		
	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65					70		
40	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
45	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110					115		
	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 312]						
50			120					125							
	PEPTIDE #B51 Met-Ala-(15-125)hIL-3 pMON13382														
55	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
	Lys	Arg	Pro	Pro	Ala	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ala
			30					35					40		
60	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn

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	45		50		55
	Leu Glu Ser Phe Val Arg Ala	Val Lys Asn Leu Glu Asn Ala Ser			
5	60	65	70		
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys Leu Pro Ser			
	75	80	85		
10	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile Lys Ala Gly			
	90	95	100		
	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Gln Phe Tyr Leu Trp Thr			
	105	110	115		
15	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 313]			
	120	125			
20	PEPTIDE #B52 Met-Asp-(15-125)hIL-3 pMON13476				
	Met Asp Asn Cys Ser Ile Met	Ile Asp Glu Ala Ile His His Leu			
	15	20	25		
25	Lys Arg Pro Pro Ala Pro Leu	Leu Asp Pro Asn Asn Leu Asn Ala			
	30	35	40		
	Glu Asp Val Asp Ile Leu Met	Glu Arg Asn Leu Arg Leu Pro Asn			
	45	50	55		
30	Leu Glu Ser Phe Val Arg Ala	Val Lys Asn Leu Glu Asn Ala Ser			
	60	65	70		
	Gly Ile Glu Ala Ile Leu Arg	Asn Leu Gln Pro Cys Leu Pro Ser			
	75	80	85		
35	Ala Thr Ala Ala Pro Ser Arg	His Pro Ile Ile Ile Lys Ala Gly			
	90	95	100		
40	Asp Trp Gln Glu Phe Arg Glu	Lys Leu Thr Phe Tyr Leu Val Thr			
	105	110	115		
	Leu Glu Gln Ala Gln Glu Gln	Gln [SEQ ID NO.: 314]			
	120	125			
45	PEPTIDE #B53 Met-Ala-(15-125)hIL-3 pMON13446				
50	Met Ala Tyr Pro Glu Thr Asp Tyr	Lys Asp Asp Asp Asp Lys Asn			
	-14	-10	-5	15	
	Cys Ser Ile Met Ile Asp Glu Ile Ile	His His Leu Lys Arg Pro			
	20	25	30		
55	Pro Ala Pro Leu Leu Asp Pro Asn Asn	Leu Asn Ala Glu Asp Val			
	35	40	45		
60	Asp Ile Leu Met Glu Arg Asn Leu Arg	Leu Pro Asn Leu Glu Ser			
	50	55	60		

139

Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser Gly Ile Glu
65 70 75

5 Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser Ala Thr Ala
80 85 90

Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly Asp Trp Gln
95 100 105

10 Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr Leu Glu Gln
110 115 120

Ala Gln Glu Gln Gln [SEQ ID NO.: 315]
125

15

PEPTIDE #B54 Met-Ala-(15-125)hIL-3 pMON13390

20 Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys Asn
-14 -10 -5 15

Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu Lys Arg Pro
20 25 30

25 Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser Glu Asp Met
35 40 45

Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn Leu Leu Ala
50 55 60

30 Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser Gly Ile Glu
65 70 75

Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser Ala Thr Ala
80 85 90

35 Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly Asp Trp Gln
95 100 105

40 Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr Leu Glu Gln
110 115 120

Ala Gln Glu Gln Gln [SEQ ID NO.: 316]
125

45

PEPTIDE #C-2 Met-Ala-(15-125)hIL-3 pMON13400

50 Met Ala Asn Cys Ser Ile Met Pro Asp Glu Ala Ile His His Leu
15 20 25

Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
30 35 40

55 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

140

75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

5 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 317]
120 125

PEPTIDE #C-3 Met-Ala-(15-125)hIL-3 pMON13402

15 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu
15 20 25

Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
30 35 40

20 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

35 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 318]
120 125

40 PEPTIDE #C-10 Met-Ala-(15-125)hIL-3 pMON13440

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

45 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
30 35 40

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

50 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Pro Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg Thr Pro Ile Ile Ile Lys Ala Gly
90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

141

	105		110		115
	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 319]				
	120		125		
5					
	PEPTIDE #C-11 Met-Ala-(15-125)hIL-3 pMON13451				
10	Met Ala Asn Cys Ser Ile Ile Leu Asp Glu Ala Ile His His Leu				
	15		20		25
	Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser				
	30		35		40
15	Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn				
	45		50		55
	Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser				
	60		65		70
20	Gly Ile Glu Pro Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser				
	75		80		85
	Ala Thr Ala Ala Pro Ser Arg Thr Pro Ile Ile Ile Lys Ala Gly				
	90		95		100
25	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr				
	105		110		115
30	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 320]				
	120		125		
	PEPTIDE #C-4 Met-Ala-(15-125)hIL-3 pMON13403				
35	Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu				
	15		20		25
	Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser				
	30		35		40
	Glu Asp Met Asp Ile Leu Met Asp Ser Asn Leu Arg Thr Pro Asn				
	45		50		55
45	Leu Leu Ala Phe Pro His Ala Ser Lys Gln Leu Glu Asn Ala Ser				
	60		65		70
	Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser				
	75		80		85
50	Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly				
	90		95		100
	Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr				
	105		110		115
55	Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 321]				
	120		125		
60					
	PEPTIDE #C-5 Met-Ala-(15-125)hIL-3 pMON13411				

143

		45		50		55									
	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		
5															
	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
10	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
	Asp	Trp	Gln	Glu	Phe	Arg	Leu	Lys	Leu	Gln	Phe	Tyr	Leu	Ser	Ser
			105					110					115		
15	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 324]						
			120					125							

PEPTIDE #C-8 Met-Ala-(15-125)hIL-3 pMON13419

20															
	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
25	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
	Glu	Asp	Met	Asp	Ile	Leu	Met	Asp	Ser	Asn	Leu	Leu	Thr	Pro	Asn
			45					50					55		
30	Leu	Leu	Ala	Phe	Pro	His	Ala	Ser	Lys	Gln	Leu	Glu	Asn	Ala	Ser
			60					65					70		
35	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80					85		
	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95					100		
40	Asp	Trp	Gln	Glu	Phe	Arg	Leu	Lys	Leu	Gln	Phe	Tyr	Leu	Ser	Ser
			105					110					115		
	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 325]						
			120					125							

PEPTIDE #C-1 Met-Ala-(15-125)hIL-3 pMON13418

50	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
55	Glu	Asp	Met	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
60	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		

Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
75 80 85
5 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly
90 95 100
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115
10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 326]
120 125

15 PEPTIDE #C-9 Met-Ala-(15-125)hIL-3 pMON13428

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25
20 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55
25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70
30 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
75 80 85
Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly
90 95 100
35 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr
105 110 115
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 327]
120 125
40

PEPTIDE #C-12 Met-Ala-(15-125)hIL-3 pMON13459

45 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu
15 20 25
50 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
30 35 40
Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55
55 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70
Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
75 80 85
60 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly

145

		90		95		100
5	Asp Trp	Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr	105	110	115	
	Leu Glu	Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 328]	120	125		
10	PEPTIDE #C-13 Met-Ala-(15-125)hIL-3 pMON13467					
15	Met Ala	Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu	15	20	25	
	Lys Ile	Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser	30	35	40	
20	Glu Asp	Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn	45	50	55	
	Leu Leu	Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser	60	65	70	
25	Gly Ile	Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser	75	80	85	
30	Ala Thr	Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly	90	95	100	
	Asp Trp	Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Ser	105	110	115	
35	Leu Glu	Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 329]	120	125		
40	PEPTIDE #C-14 Met-Ala-(15-125)hIL-3 pMON13492					
45	Met Ala	Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu	15	20	25	
	Lys Ile	Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser	30	35	40	
50	Glu Asp	Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn	45	50	55	
	Leu Leu	Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser	60	65	70	
55	Gly Ile	Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr	75	80	85	
	Trp Thr	Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly	90	95	100	
60	Asp Trp	Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr				

5

TABLE 4
DNA SEQUENCES

5	pMON13287
	Met-Ala- (15-125) IL-3
	DNA sequence #1
10	ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT G
	CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC A
15	AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT T
	CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA G
20	GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA G
25	GAACAACAG [SEQ ID NO:97]
	pMON13290
30	Met-Ala- (15-125) IL-3
	DNA sequence #2
	ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT G
35	CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC A
	AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT T
40	CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA G
45	GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA G
	GAACAACAG [SEQ ID NO:98]
50	
	pMON13313
	Met-Ala- (15-125) IL-3
55	DNA sequence #3
	ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT G
60	CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
5 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
10 GAACAACAG [SEQ ID NO:99]

pMON13288
15 **Met-Ala- (15-125) IL-3**
DNA sequence #4
20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G
CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO:100]

pMON13312
40 **Met-Ala- (15-125) IL-3**
DNA sequence #5
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G
45 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
50 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
55 GAACAACAG [SEQ ID NO:101]

pMON13294
60 **Met-Ala- (15-125) IL-3**

DNA sequence #6

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G
5 CTGGACCCGAACACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
10 T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:102]

20
pMONM13289

Met-Ala- (15-125) IL-3

DNA sequence #7

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G
30 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
35 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
40 GAACAACAG [SEQ ID NO:103]

45
pMON13292

Met-Ala- (15-125) IL-3

DNA sequence #8

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G
50 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
55 T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
60 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

150

G

GAACAACAG [SEQ ID NO:104]

5

pMON13295

Met-Ala-(15-125) IL-3

10 **DNA sequence #9**

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G

15

CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

20

CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G

25

GAACAACAG [SEQ ID NO:105]

30

pMON13344

(15-125) IL-3

DNA sequence #10

35 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

40

AACCTCGAGGCATTCAACCGTCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATCT
T

AAAAATCTCCTGCCATGTCTGCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

45

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGAGAGACGCGCA
G

50

GCTCAACAG [SEQ ID NO:106]

pMON13345

55

(15-125) IL-3

DNA sequence #11

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

60

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

151

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

5 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

10 GCTCAACAG [SEQ ID NO:107]

pMON13346

15 **(15-125) IL-3**

DNA sequence #12

20 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTCCACCTGCACCTTTG
CTGGACAGTAACAACCTCAATCCGAAGACATGGATATCCTGATGAAAATAACCTTCGTCGTCC
A

25 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

30 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:108]

35

pMON13347

40 **(15-125) IL-3**

DNA sequence #13

45 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCC
A

50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

55 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:109]

60

pMON13348

152

(15-125) IL-3

DNA sequence #14

5 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCC
10 A
AACCCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTTCT
T
15 AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G
GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G
20 GCTCAACAG [SEQ ID NO:110]

pMON13349

(15-125) IL-3

DNA sequence #15

30 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGAACTCC
A
35 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G
40 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G
GCTCAACAG [SEQ ID NO:111]

pMON13350

(15-125) IL-3

DNA sequence #16

50 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCTCC
55 A
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
60 CGTAATCTCCAACCATGTCTGCCCCCTGCCACGGCCGCACCCCTCTCGACATCCAATCATCATCAA
G

153

GCAGGTGACTGGCAAGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

5 GCTCAACAG [SEQ ID NO:112]

pMON13355

10 **(15-125) IL-3**

DNA sequence #17

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
15 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCC
A

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
20 T

CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G

GCAGGTGACTGGCAAGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
25 G

GCTCAACAG [SEQ ID NO:113]

30

pMON13352

(15-125) IL-3

35 **DNA sequence #18**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCC
40 A

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
45 G

GACGGTGACTGGAATGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50

GAACAACAG [SEQ ID NO:114]

pMON13354

55

(15-125) IL-3

DNA sequence #19

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
60

154

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
5 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T
AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G
10 GACGGTGACTGGAATGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:115]
15
pMON13363
(15-125) IL-3 SECRETED
20 **DNA sequence #20**
AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
25 A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
30 AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
G
35 GCTCAACAG [SEQ ID NO:116]

pMON13364
40 **(15-125) IL-3 SECRETED**
DNA sequence #21
45 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG
CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G
55 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
G
GCTCAACAG [SEQ ID NO:117]
60

155

pMON13365

(15-125) IL-3 SECRETED

5 DNA sequence #22

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTCCACCTGCACCTTTG
 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
 10 A
 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
 T
 15 AAAAATCTCCTGCCATGTCTGCCCCGTCACGCGACCCACGCGACATCCAATCCATATCAA
 G
 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
 G
 20 GCTCAACAG [SEQ ID NO:118]

pMON13360

25 (15-125) IL-3 SECRETED

DNA sequence #23

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCC
 A
 35 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
 T
 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCCTCTCGACATCCAATCATCATCAA
 G
 40 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
 G
 GAACAACAG [SEQ ID NO:119]
 45

pMON13361

50 (15-125) IL-3 SECRETED

DNA sequence #24

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCC
 A
 55 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
 T
 60 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCCTCTCGACATCCAATCACCATCAA

G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
5 GAACAACAG [SEQ ID NO:120]

pMON13362
10 **(15-125) IL-3 SECRETED**
DNA sequence #25
15 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
20 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGCCCGCACCTCTCGACATCCAATCACCATCAA
G
25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:121]
30

pMON13301
35 **(15-125) IL-3 INTRACELLULAR**
DNA sequence #26
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
40 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
45 AAAAATCTCCTGCCATGTCTGCCCCTGCCACGCCCGCACCCACGCGACATCCAATCCATATCAA
G
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
50 G
GCTCAACAG [SEQ ID NO:122]

pMON13302
55 **(15-125) IL-3 INTRACELLULAR**
DNA sequence #27
60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

10 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:123]

15

pMON13303

(15-125) IL-3 INTRACELLULAR

20 **DNA sequence #28**
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTCCACCTGCACCTTT
G

25 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGCAATTGAGAGCATTCT
T

30 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

35 GCTCAACAG [SEQ ID NO:124]

40 **pMON13298**

(15-125) IL-3 INTRACELLULAR

DNA sequence #29

45 ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
G

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCTCC
A

50 AACCTCGAGGCATTCAACCGTGTCTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

60 GAACAACAG [SEQ ID NO:125]

158

pMON13299

(15-125) IL-3 INTRACELLULAR

5 **DNA sequence #30**
ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
G
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGTCC
10 A
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
15 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
G
20 GAACAACAG [SEQ ID NO:126]

pMON13300

Met-Ala- (15-125) IL-3 INTRACELLULAR

25 **DNA sequence #31**
ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
30 G
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGTCC
A
35 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:127]
45 **DNA sequence #32**
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G
50 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACCTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCT
T
55 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
60 G

GAACAACAG [SEQ ID NO: 160]

5 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

5 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACATGGACATTTGATGGAACGAAACCTTCGAACTCCA

10 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

15 G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO: 161]

20 DNA sequence #B1 pMON13406 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

25 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCTCTCGACATCCAATCATCATCAA

35 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 332]

40 DNA sequence #B2 pMON13414 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

45 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

50 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCCTCTCGACATCCAATCATCATCAA

55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 333]

60 DNA sequence #B3 pMON13407 Met-Ala-(15-125) IL-3

160

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

5 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
15 G

GAACAACAG [SEQ ID NO.: 334]

20 DNA sequence #B4 pMON13405 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

25 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
35 G

GAACAACAG [SEQ ID NO.: 335]

40 DNA sequence #B5 pMON13415 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAATGATCCATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

45 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
55 G

GAACAACAG [SEQ ID NO.: 336]

60 DNA sequence #B6 pMON13408 Met-Ala-(15-125) IL-3

161

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

5 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATCGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 337]

DNA sequence #B7 pMON13409 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGCTGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 338]

DNA sequence #B8 pMON13410 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGGACGAACGAAACCTTCGAACTCC
A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 339]

DNA sequence #B9 pMON13422 Met-Ala-(15-125) IL-3

60 ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACTCC

5 A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

10 G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

G

15 GAACAACAG [SEQ ID NO.: 340]

DNA sequence #B10 pMON13423 Met-Ala-(15-125) IL-3

20 ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACTCC

A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

30 G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

G

35 GAACAACAG [SEQ ID NO.: 341]

DNA sequence #B11 pMON13424 Met-Ala-(15-125) IL-3

40 ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACTCC

A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

50 G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

G

55 GAACAACAG [SEQ ID NO.: 342]

DNA sequence #B12 pMON13425 Met-Ala-(15-125) IL-3

60 ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

G

163

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 343]

15

DNA sequence #B13 pMON13426 Met-Ala-(15-125) IL-3

20 ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGGACGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 344]

35

DNA sequence #B14 pMON13429 Met-Ala-(15-125) IL-3

40 ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTATATCCTGGACGAACGAAACCTTCGAACTCCA

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

50 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 345]

55

DNA sequence #B15 pMONM13368 Met-Ala-(15-125) IL-3

60 ATGGCTAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTT
G

CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC

164

A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

5 T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

10 G

GAACAACAG [SEQ ID NO.: 346]

15 DNA sequence #B16 pMON13380 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTT

G

20 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC

A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

T

25 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA

30 G

GAACAACAG [SEQ ID NO.: 347]

35 DNA sequence #B17 pMON13475 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT

G

40 CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC

A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

T

45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

50 G

GAACAACAG [SEQ ID NO.: 348]

55 DNA sequence #B18 pMON13366 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT

G

60 CTGGACCCGAACAACCTCAATAACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC

A

165

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO.: 349]

DNA sequence #B19 pMON13367 Met-Ala-(15-125) IL-3

15 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

30 GAACAACAG [SEQ ID NO.: 350]

DNA sequence #B20 pMON13369 Met-Ala-(15-125) IL-3 42D, 46S, 50D

35 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50 GAACAACAG [SEQ ID NO.: 351]

DNA sequence #B21 pMON13370 Met-Ala-(15-125) IL-3

55 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

166

T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

5 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 352]

10 DNA sequence #B22 pMON13373 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

15 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 353]

30 DNA sequence #B23 pMON13374 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

35 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 354]

50 DNA sequence #B24 pMON13375 Met-Ala-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

55 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

5 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAG [SEQ
ID NO.: 355]

DNA sequence #B25 pMON13376 Met-Asp-(15-119) IL-3

10 ATGGATAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
15 A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 356]

25 DNA sequence #B26 pMON13377 Met-Ala-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
30 G

CTGGACCCGAACAACCTCAATGACGAAGACGTCCTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

35 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

40 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 357]

45 DNA sequence #B27 pMON13378 Met-Asp-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

50 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

55 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 358]

60

DNA sequence #B28 pMON13379 Met-Ala-(15-125) IL-3

5 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATCCTGATGGACCGAAACCTTCGACTTCC
A
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 359]
20

DNA sequence #B29 pMON13385 Met-Ala-(15-125) IL-3

25 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTACCACCTCGCCCTTC
C
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
30 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
35 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 360]
40

DNA sequence #B30 pMON13381 Met-Ala-(15-125) IL-3

45 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
50 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 361]
60

169

DNA sequence #B31 pMON13383 Met-Ala-(15-125) IL-3

5 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTTCTGATGGACCGAAACCTTCGACTTCC
A
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 362]

20 DNA sequence #B32 pMON13384 Met-Ala-(15-125) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
25 G
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
30 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
35 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 363]

40 DNA sequence #B33 pMON13388 Met-Ala-(15-125) IL-3
ATGGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
45 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGACCGAAACCTTCGACTTAG
C
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
50 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 364]

60 DNA sequence #B34 pMON13389 Met-Ala-(15-125) IL-3

170

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

5 CTGGACCCGAACAACCTCAATGACGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 365]

20 DNA sequence #B35 pMON13391 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTC
C

25 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 366]

40 DNA sequence #B36 pMON13392 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

45 CTGGACCCGAACAACCTCAATGACGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 367]

60 DNA sequence #B37 pMON13393 Met-Ala-(15-125) IL-3

171

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C

5 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 368]

DNA sequence #B38 pMON13394 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

25 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 369]

DNA sequence #B39 pMON13395 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTC
C

CTGGACCCGAACAACCTCAATGACGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

45 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 370]

DNA sequence #B40 pMON13396 Met-Ala-(15-125) IL-3

60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT

G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

5 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCG
T

10 ATGGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 371]

DNA sequence #B41 pMON13397 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

25 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCG
T

30 ATGGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 372]

DNA sequence #B42 pMON13398 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTCCTATCCTGATGGAACGAAACCTTCGACTTCC
A

45 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

50 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 373]

DNA sequence #B43 pMON13399 Met-Ala-(15-125) IL-3

60 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTC
C

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO.: 377]

DNA sequence #B47 pMON13287 Met-Ala-(15-125) IL-3

15 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTCATGTCTCTGATGGAACGAAACCTTCGACTTCC
A

20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

25 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

30 GAACAACAG [SEQ ID NO.: 378]

DNA sequence #B48 pMON13420 Met-Ala-(15-125) IL-3

35 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGACCTTCC
CTGGACCCGAACAACCTCAATGACGAAGACGTCCTATCCTGATGGACCGAAACCTTCACTTAGC

40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 379]

50 DNA sequence #B49 pMON13421 Met-Ala-(15-125) IL-3

55 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C

CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTAG
C

60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

- CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG
- 5 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
- GAACAACAG [SEQ ID NO.: 380]
- 10 DNA sequence #B50 pMON13432 Met-Ala-(15-125) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C
- 15 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTCC
A
- AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
- 20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG
G
- GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
- 25 GAACAACAG [SEQ ID NO.: 381]
- 30 DNA sequence #B51 pMON13382 Met-Ala-(15-125) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
- 35 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
- AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
- 40 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG
G
- GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGTGGACCCTTGAGCAAGCGCA
G
- 45 GAACAACAG [SEQ ID NO.: 382]
- 50 DNA sequence #B52 pMON13476 Met-Asp-(15-125) IL-3
ATGGATAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G
- 55 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
- AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
- 60 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG

DNA sequence #C10

ATGGCTAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCCTAG
C

5 CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO:386]

20 pMON13451
Met-Ala-(15-125) IL-3
DNA sequence #C11

25 ATGGCTAACTGCTCTATAATACTCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

35 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

40 GAACAACAG [SEQ ID NO:387]

45 pMON13403
Met-Ala-(15-125) IL-3
DNA sequence #C4

50 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCC
A

55 AACCTGCTCGCATTCACACATGCTGTCAAGCAATTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

60

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

5 GAACAACAG [SEQ ID NO:388]

pMON13419

Met-Ala-(15-125) IL-3

10 DNA sequence #C8

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

15 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCC
A

AACCTGCTCGCATTTCCACATGCTTCTAAGCAATTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:389]

30 pMON13411

Met-Ala-(15-125) IL-3

DNA sequence #C5

35 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

40 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
G

50 GAACAACAG [SEQ ID NO:390]

pMON13412

55 Met-Ala-(15-118) IL-3

DNA sequence #C6

60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

179

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTTAATA
[SEQ ID NO:391]

15 pMON13413
Met-Ala-(15-125) IL-3
DNA sequence #C7

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO:392]

40 pMON13418
Met-Ala-(15-125) IL-3
DNA sequence #C1

45 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

60 GAACAACAG [SEQ ID NO:393]

180

pMON13428

Met-Ala-(15-125) IL-3

5

DNA sequence #C9

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

10

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

15

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

20

GCAGGTGACTGGCAAGAATTCCGGCTTAAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:394]

25

pMON13459

Met-Ala-(15-125) IL-3

30

DNA sequence #C12

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCTAG
C

35

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

40

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
G

45

GAACAACAG [SEQ ID NO:395]

50

pMON13467

Met-Ala-(15-125) IL-3

DNA sequence #C13

55

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

60

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO:396]

pMON13492

15 Met-Ala- (15-125) IL-3

DNA sequence #C14

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:397]

35 pMON13446

40 Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-Ala (15-
125) IL-3

DNA sequence #B53

45 ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGA
T

GAAATTATACATCACTTAAAGAGACCACCTGCACCTTTGCTGGACCCGAACAACCTCAATGCTGA
A

50 GACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCAAACCTGGAGAGCTTCGTAAGGGCTGT
C

AAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTC
T

55 GCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGA
A

AACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:404]

60

pMON13390

Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-Ala (15-125) IL-3

5

DNA sequence #B54

ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGA
T

10

GAAATTATACATCACTTAAAGAGACCACCTAACCCTTTGCTGGACCCGAACAACCTCAATTCCGA
A

15

GACATGGATATCCTGATGGAACGAAACCTTCGAACTCCAAACCTGCTCGCATTCGTAAGGGCTGT
C

AAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTC
T

20

GCCACGGCCGCACCCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGA
A

AAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:405]

25

Polypeptides corresponding to [SEQ ID NO. 129] comprising (1-133)hIL-3 containing four or more amino acid substitutions can be made using the procedures described above and in the following examples by starting with the appropriate oligonucleotides and then constructing the DNA encoding the polypeptide and expressing it in an appropriate host cell. In a similar manner polypeptides which correspond to [SEQ ID NO. 130] and contain four or more amino acid substitutions and wherein from 1 to 14 amino acids have been sequentially deleted from the N-terminus, or from 1 to 15 amino acids have been deleted from the C-terminus or deletions of amino acids have been made from both the N-terminus and the C-terminus can also be made by following the procedures described above and in the following examples, beginning with the appropriate starting materials.

Further details known to those skilled in the art may be found in T. Maniatis, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor

Laboratory (1982) and references cited therein,
 incorporated herein by reference; and in J. Sambrook,
 et al., Molecular Cloning, A Laboratory Manual, 2nd
 edition, Cold Spring Harbor Laboratory (1989) and
 5 references cited therein, incorporated herein by
 reference.

The following examples will illustrate the
 invention in greater detail although it will be
 understood that the invention is not limited to these
 10 specific examples.

Amino acids are shown herein by standard one
 letter or three letter abbreviations as follows:

15	Abbreviated Designation		Amino Acid
	A	Ala	Alanine
	C	Cys	Cysteine
20	D	Asp	Aspartic acid
	E	Glu	Glutamic acid
	F	Phe	Phenylalanine
	Abbreviated Designation		Amino Acid
25	G	Gly	Glycine
	H	His	Histidine
	I	Ile	Isoleucine
	K	Lys	Lysine
30	L	Leu	Leucine
	M	Met	Methionine
	N	Asn	Asparagine
	P	Pro	Proline
	Q	Gln	Glutamine
35	R	Arg	Arginine
	S	Ser	Serine
	T	Thr	Threonine
	V	Val	Valine

- Biesma, B. et al., Effects of interleukin-3 after chemotherapy for advanced ovarian cancer. Blood, 80:1141-1148 (1992).
- 5 Birnboim, H. C. and J. Doly. A rapid alkaline extraction method for screening recombinant plasmid DNA. Nucleic Acids Research, 7(6): 1513-1523 (1979).
- 10 Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, 72: 248-254 (1976).
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10 EXAMPLE 1
Construction of pMON 5846 (Fig. 4) which encodes [Met-
(1-133)hIL-3 (Arg¹²⁹)]

A plasmid containing the gene for the cDNA of
 hIL-3 cloned into pUC18 on an EcoRI to HindIII
 15 fragment was obtained from British Biotechnology
 Limited (Cambridge, England). This plasmid was
 designated pPO518. The purified plasmid DNA was
 cleaved by the restriction endonucleases NheI and
 BamHI. Approximately 0.5 micrograms of cleaved
 20 plasmid DNA was ligated to 1.0 picomoles of a pair of
 annealed oligonucleotides with the following sequence:

5'-CTAGCGATCTTTTAATAAGCTTG-3' [SEQ ID NO: 1]
 3'-GCTAGAAAATTATTCGAACCTAG-5' [SEQ ID NO: 2]

25 The ligation mixture was used to transform
 competent JM101 cells to ampicillin resistance.
 Colonies were picked, and plasmid DNA was purified and
 subjected to restriction enzyme analysis. An isolate
 30 was identified in which the above oligonucleotide
 sequence had replaced the portion of the gene that
 encodes the extreme C terminus. Within the new
 sequence was a new stop codon, TAA, and a recognition
 site for the enzyme HindIII. The new plasmid was
 35 designated pMON5846.

EXAMPLE 2

(a) Construction of expression vector plasmid pMON2341

5 The plasmid pMON2341 was used to supply the particular replicon and expression elements used for construction of many of the plasmids used to produce hIL-3 and hIL-3 muteins in E. coli. These expression elements are described in the materials and methods
10 section. pMON2341 is derived from pMON5515 (Olins et al., 1988) and from pMON2429. pMON2429 consists of the phage mp18 (Yanisch-Perron et al., 1985) with a BclI fragment carrying the chloramphenicol acetyl transferase (cat) gene from pBR328 (Covarrubias et
15 al., 1981) inserted into the BamHI site. The cat gene in pMON2429 has been altered from that in pBR328 by site directed mutagenesis (Kunkel, 1985). The recognition sites for NcoI and EcoRI which occur in the native gene were altered so that these two
20 restriction enzymes no longer recognize these sites. The changes did not alter the protein specified by the gene. Also, an NcoI site was introduced at the N-terminus of the coding sequence so that it overlaps the codon for initiator methionine.

25 The steps involved in construction of pMON2341 are listed below:

(1) The DNAs of pMON5515 and pMON2429 were treated with NcoI and HindIII. The fragments were ligated and used to transform competent E. coli to
30 ampicillin resistance. From these colonies, some were identified that were chloramphenicol resistant. From one of these colonies, plasmid DNA was isolated in which the rat atriopeptigen gene of pMON5515 had been replaced by the NcoI to HindIII fragment containing
35 the cat gene from pMON2429. This fragment contains the recognition sites for several restriction enzymes

in the portion derived from the multilinker region of mp18. The new plasmid was designated pMON2412.

(2) pMON2412 was treated with the enzyme ClaI which cleaves at one location in the pBR327 derived portion of the DNA. The protruding ends were rendered blunt by treatment with Klenow in the presence of nucleotide precursors. This DNA was mixed with an isolated 514 bp RsaI fragment derived from pEMBL8 (Dente et al., 1983). This RsaI fragment contains the origin of replication of phage f1. This ligation mixture was used to transform competent *E. coli* cells to ampicillin resistance. Among the plasmid DNAs isolated from these cells was pMON5578. This plasmid has the structure of pMON2412 with the f1 origin region inserted into the ClaI site. This is illustrated in the Figures and in Olins and Rangwala (1990).

(3) The DNA of pMON5578 was treated with restriction enzymes HindIII and MstII. The DNA was then treated with Klenow enzyme in the presence of nucleotide precursors to render the ends blunt. This treated DNA was ligated and used to transform competent *E. coli* to ampicillin resistance. From the ampicillin resistant colonies, one plasmid was recovered from which the portion between HindIII and MstII was absent. This deletion resulted in the removal of sequences from the plasmid which are recognized by a number of restriction endonuclease sites. The new plasmid was designated pMON5582.

(4) The DNA of pMON5582 was treated with SstII and BclII and ligated in the presence of annealed oligonucleotides with the sequences shown below.

5'- GGCAACAATTTCTACAAAACACTTGATACTGTATGAGCAT-
3'-CGCCGTTGTAAAGATGTTTTGTGAACATATGACATACTCGTA-

ACAGTATAATTGCTTCAACAGAACAGATC-3' [SEQ ID NO:3]
TGTCATATTAACGAAGTTGTCTTGT-5' [SEQ ID NO:4]

This sequence encodes the essential elements of
5 the *recA* promoter of *E. coli* including the
transcription start site and the *lexA* repressor
binding site (the operator) (Sancar et al., 1980).
The plasmid recovered from the ligation mixes
10 contained this *recA* promoter in place of the one in
pMON5582 (and in pMON5515). The functionality of the
recA promoter was illustrated by Olins and Rangwala
(1990). The new plasmid was designated pMON5594.

(5) To eliminate the single *EcoRI* site in
pMON5594, the DNA was treated with *EcoRI*, then with
15 Klenow in the presence of nucleotide precursors to
render the ends blunt and then the DNA was ligated.
From this ligation mix a plasmid was recovered whose
DNA was not cleaved with *EcoRI*. This plasmid was
designated pMON5630.

20 (6) To alter the single recognition site for
PstI, plasmid pMON5630 was subjected to site directed
mutagenesis (Kunkel, 1985). The oligonucleotide used
in this procedure has the sequence shown below.

25 5'-CCATTGCTGCCGGCATCGTGGTC-3' [SEQ ID NO:5]

The result of the procedure was to construct
pMON2341 which differs from pMON5630 in that the *PstI*
site in the beta-lactamase gene was altered so that
30 *PstI* no longer recognizes the site. The single
nucleotide change does not alter the amino acid
sequence of the beta-lactamase protein.

(b) Construction of pMON5847 (Fig. 5) which encodes
[Met-(1-133)hIL-3(Arg129)]

35 Plasmid pMON2341 was used to supply the replicon,
promotor, ribosome binding site, transcription

terminator and antibiotic resistance marker for the plasmids used to produce hIL-3 in *E. coli* from cDNA derived hIL-3 genes.

Plasmid pMON2341 was treated with restriction enzymes NcoI and HindIII. The restriction fragment containing the replication origin was purified. The DNA of plasmid pMON5846 was treated with NcoI and HindIII. The restriction fragment containing the hIL-3 gene was gel purified. These purified restriction fragments were mixed and ligated. The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and analyzed using restriction enzymes. pMON5847 was identified as a plasmid with the replicon of pMON2341 and the hIL-3 gene in place of the chloramphenicol acetyl transferase gene. JM101 cells harboring this plasmid were cultured in M9 medium and treated with nalidixic acid as described above. Samples of the culture were examined for protein content. It was found that this hIL-3 mutein was produced at about 6% of total cell protein as measured on Coomassie stained polyacrylamide gels.

25

EXAMPLE 3

Construction of pMON5854 (Fig. 7) which encodes [Met-(1-133)hIL-3(Arg129)]

To increase the accumulation of hIL-3 in *E. coli*, the coding sequence of the amino terminal portion of the protein was altered to more closely reflect the codon bias found in *E. coli* genes that produce high levels of proteins (Gouy and Gautier, 1982). To change the coding sequence for the amino terminal portion of the gene, a pair of synthetic oligonucleotides were inserted between the NcoI and HpaI sites within the coding sequence. About 0.5

micrograms of DNA of the plasmid pMON5847 (Example 2) was treated with NcoI and HpaI. This DNA was mixed with an annealed pair of oligonucleotides with the following sequence:

5

5'-CATGGCTCCAATGACTCAGACTACTTCTCTTAAGACT-
3'-CGAGGTTACTGAGTCTGATGAAGAGAATTCTGA-

TCTTGGGTT-3' [SEQ ID NO:6]

10 AGAACCCAA-5' [SEQ ID NO:7]

The fragments were ligated. The ligation mixture was used to transform competent JM101 to ampicillin resistance. Colonies were picked into broth. From the cultures plasmid DNA was made and examined for the presence of a DdeI site (CTNAG) which occurs in the synthetic sequence but not between the NcoI and HpaI sites in the sequence of pMON5847. The new recombinant plasmid was designated pMON5854. The nucleotide sequence of the DNA in the coding sequence of the amino terminal portion of the hIL-3 gene in pMON5854 was determined by DNA sequencing and found to be the same as that of the synthetic oligonucleotide used in ligation. Cultures of JM101 cells harboring this plasmid were grown and treated with nalidixic acid to induce production of the hIL-3 mutant protein. Analysis of the proteins on Coomassie gels showed that the accumulation of hIL-3 mutein was about 25% of total cell protein in cultures harboring pMON5854, significantly higher than it was in cultures harboring pMON5847.

EXAMPLE 4

Construction of pMON5887 (Fig. 12) which encodes [Met-(1-125)hIL-3]

The plasmid DNA of pMON5854 (Example 3) was

	Met	Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	Lys	Thr	Ser
	Trp	Val	Asn	Cys	Ser	Asn	Met	Ile	Asp	Glu	Ile	Ile	Thr
	His	Leu	Lys	Gln	Pro	Pro	Leu	Pro	Leu	Leu	Asp	Phe	Asn
30	Asn	Leu	Asn	Gly	Glu	Asp	Gln	Asp	Ile	Leu	Met	Glu	Asn
	Asn	Leu	Arg	Arg	Pro	Asn	Leu	Glu	Ala	Phe	Asn	Arg	Ala
	Val	Lys	Ser	Leu	Gln	Asn	Ala	Ser	Ala	Ile	Glu	Ser	Ile
	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro	Leu	Ala	Thr	Ala
	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp	Gly	Asp
35	Trp	Asn	Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys
	Thr	Leu	Glu	Asn	Ala	Gln	Ala	Gln	Gln	{SEQ ID NO:10}			

EXAMPLE 5

Construction of pMON5967 which encodes [Met-Ala-(15-125)hIL-3]

5 Plasmid DNA of pMON5887 isolated from E. coli GM48 (dam-) was cleaved with NcoI and ClaI and ligated to 1 picomole of an annealed pair of oligonucleotides, encoding amino acids [Met Ala (15-20)hIL-3]. The
10 sequence of these oligonucleotides is shown below.
5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:11]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:12]

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant
15 colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be smaller than that of pMON5887 by restriction analysis using NcoI and NsiI. The nucleotide sequence of the region between NcoI and ClaI was determined and
20 found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5967 and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein purification and bio-assay.

25

EXAMPLE 6

Construction of pMON5978 which encodes [Met-Ala-(15-125)hIL-3]

30 Plasmid DNA of pMON5967 isolated from E. coli GM48(dam-) was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six oligonucleotides encoding hIL-3 amino acids 20-70 (FIG. 2). This synthetic fragment encodes three
35 unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in

from pMON5988 was ligated to the following annealed complementary oligonucleotides from Table (2).

Oligo #13 [SEQ ID NO:27]

5 **Oligo #14** [SEQ ID NO:28]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates.

Plasmid DNA was isolated from a colony grown in LB
10 broth and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 99 bases between the NcoI and EcoRV restriction sites in the (15-125) hIL-3 gene
15 are replaced with 22 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

20 EXAMPLE 8

Construction of pMON13344

Plasmid pMON13356 DNA was digested with restriction enzymes NcoI and EcoRV, and the resulting 4190 base pair NcoI,EcoRV fragment contains the following
25 genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 47-125 of (15-125)hIL-
30 3. The second DNA fragment was generated by synthetic gene assembly using the following complementary oligonucleotide pairs that have overlapping ends:

Oligo #1 [SEQ ID NO:15]

Oligo #2 [SEQ ID NO:16]

Oligo #3 [SEQ ID NO:17]

5 **Oligo #4** [SEQ ID NO:18]

Oligo #9 [SEQ ID NO:23]

Oligo #10 [SEQ ID NO:24]

The assembled oligonucleotides create NcoI and EcoRV
 10 restriction ends and the DNA sequence that encodes
 amino acids 15-46 of (15-125)hIL-3 with the following
 amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A
 and 45V. The codons encoding amino acids 15-46 of
 (15-125)hIL-3 are those found in the hIL-3 cDNA
 15 sequence except at those positions where amino acid
 substitutions were made. The 4190 base pair
 NcoI,EcoRV restriction fragment from pMON13356 was
 ligated with the pairs of annealed oligonucleotides.
 The ligation reaction was digested with NdeI and
 20 subsequently used to transform E. coli K-12 strain
 JM101. Transformant bacteria were selected on
 ampicillin-containing plates. Plasmid DNA was
 isolated from a colony grown in LB broth. The DNA
 sequence was determined to be that of the
 25 oligonucleotides. The plasmid, pMON13344, encodes the
 (15-125)hIL-3 variant with the following amino acid
 sequence:

Peptide #2

30 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 35 Glu Asp Val Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 40 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 5 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 10 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:66]

DNA sequence #10 [SEQ ID NO:106] codes for the
 foregoing pMON13344 polypeptide.

EXAMPLE 9

15 Construction of pMON13345

The 4190 base pair NcoI, EcoRV restriction fragment
 from pMON13356 was ligated with the following pairs of
 annealed complementary oligonucleotides:

Oligo #1 [SEQ ID NO:15]
 20 **Oligo #2** [SEQ ID NO:16]

Oligo #5 [SEQ ID NO:19]
Oligo #6 [SEQ ID NO:20]

 25 **Oligo #11** [SEQ ID NO:25]
Oligo #12 [SEQ ID NO:26]

The assembled oligonucleotides create NcoI and EcoRV
 restriction ends and the DNA sequence that encodes
 30 amino acids 15-46 of (15-125)hIL-3 with the following
 amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S
 and 45M. The codons encoding amino acids 15-46 of
 (15-125)hIL-3 are those found in the hIL-3 cDNA
 sequence except at those positions where amino acid
 35 substitutions were made. The ligation reaction was
 digested with NdeI and used to transform E. coli K-12
 strain JM101. Transformant bacteria were selected on
 ampicillin-containing plates. Plasmid DNA was
 isolated from a colony grown in LB broth. The DNA was
 40 sequenced to determine that the sequence was that of

the oligonucleotides. The plasmid, pMON13345, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #3

5 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
10 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
15 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
20 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:67]

DNA sequence #11 [SEQ ID NO:107] codes for the foregoing pMON13345 polypeptide.

30

EXAMPLE 10

Construction of pMON13346

The 4190 base pair NcoI,EcoRV restriction fragment from pMON13356 was ligated with the following pairs of
35 annealed complementary oligonucleotides:

Oligo #1 [SEQ ID NO:15]

Oligo #2 [SEQ ID NO:16]

Oligo #7 [SEQ ID NO:21]

40 **Oligo #8** [SEQ ID NO:22]

Oligo #11 [SEQ ID NO:25]

Oligo #12 [SEQ ID NO:26]

45 The assembled oligonucleotides create NcoI and EcoRV

restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125)hIL-3 with the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S and 45M. The codons encoding amino acids 15-46 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and DNA sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13346, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #4

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]

DNA sequence #12 [SEQ ID NO:108] codes for the foregoing pMON13346 polypeptide.

EXAMPLE 11Construction of pMON13357

Plasmid pMON5988 DNA was digested with restriction

enzymes EcoRV and NsiI, and the resulting 4218 base pair EcoRV,NsiI fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. The 4218 base pair EcoRV,NsiI restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides:

Oligo #19 [SEQ ID NO:33]

Oligo #20 [SEQ ID NO:34]

15 The ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 was isolated from a colony grown in LB broth, and the
 size of the inserted fragment was determined by
 20 restriction analysis employing restriction enzymes
 NcoI and HindIII in double digest. In the resulting
 plasmid the 71 bases between the EcoRV and NsiI
 restriction sites in the (15-125)hIL-3 gene are
 replaced with 22 bases from the above mentioned
 25 oligonucleotides. This linker also contains a NdeI
 recognition sequence.

EXAMPLE 12

Construction of pMON13347

30 The 4218 base pair EcoRV,NsiI restriction fragment from
pMON13357 was ligated with the following pairs of
annealed complementary oligonucleotides:

Oligo #21 [SEQ ID NO:35]

35 Oligo #22 [SEQ ID NO:36]

Oligo #25 [SEQ ID NO:39]

Oligo #26 [SEQ ID NO:40]

Oligo #31 [SEQ ID NO:45]

5 **Oligo #32** [SEQ ID NO:46]

The assembled oligonucleotides create EcoRV and NsiI restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55L, 59L, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13347, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #5

25 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
40 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
45 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]

DNA sequence #13 [SEQ ID NO:109] codes for the foregoing pMON13347 polypeptide.

EXAMPLE 13

5 Construction of pMON13348

The 4218 base pair EcoRV,NsiI restriction fragment from pMON13357 was ligated with the following pairs of annealed complementary oligonucleotides:

10 **Oligo #21** [SEQ ID NO:35]

Oligo #22 [SEQ ID NO:36]

Oligo #27 [SEQ ID NO:41]

Oligo #28 [SEQ ID NO:42]

15

Oligo #31 [SEQ ID NO:45]

Oligo #32 [SEQ ID NO:46]

The assembled oligonucleotides create EcoRV and NsiI
20 restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence
25 except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated
30 from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13348, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

35 **Peptide #6**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

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Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
5 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
10 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:70]

DNA sequence #14 [SEQ ID NO:110] encodes the
foregoing pMON13348 polypeptide.

25

EXAMPLE 14

Construction of pMON13349

The 4218 base pair EcoRV, NsiI restriction fragment from
pMON13357 was ligated with the following pairs of
30 annealed complementary oligonucleotides:

Oligo #23 [SEQ ID NO:37]

Oligo #24 [SEQ ID NO:38]

35 **Oligo #25** [SEQ ID NO:39]

Oligo #26 [SEQ ID NO:40]

Oligo #29 [SEQ ID NO:43]

Oligo #30 [SEQ ID NO:44]

40

The assembled oligonucleotides create EcoRV and NsiI
restriction ends and the DNA sequence that encodes
amino acids 47-71 of (15-125)hIL-3 with the following
amino acid substitutions: 51R, 55T, 59L, 62V, 67H and

69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and the DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13349, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #7

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:71]

DNA sequence #15 [SEQ ID NO:111] encodes the foregoing pMON13349 polypeptide.

EXAMPLE 15

Construction of pMON13358

Plasmid pMON5988 DNA was digested with restriction enzymes NsiI and EcoRI and the resulting 4178 base pair NsiI, EcoRI fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of

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replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-71 and 106-125 of (15-
5 125)hIL-3. The 4178 base pair NsiI,EcoRI restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides.

Oligo #15 [SEQ ID NO:29]

10 **Oligo #16** [SEQ ID NO:30]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA
15 was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 111 bases between the NsiI and EcoRI restriction
20 sites in the (15-125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

25 EXAMPLE 16

Construction of pMON13350

The 4178 base pair NsiI,EcoRI restriction fragment from pMON13358 was ligated with the following pairs of
annealed complementary oligonucleotides:

30

Oligo #41 [SEQ ID NO:55]

Oligo #42 [SEQ ID NO:56]

Oligo #39 [SEQ ID NO:53]

35 **Oligo #40** [SEQ ID NO:54]

25 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 40 Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:72]

DNA sequence #16 [SEQ ID NO:112] codes for the foregoing pMON13350 polypeptide.

5

EXAMPLE 17

Construction of pMON13355

The 4178 base pair NsiI,EcoRI restriction fragment from pMON13358 was ligated with the following pairs of
10 annealed complementary oligonucleotides:

Oligo #41 [SEQ ID NO:55]

Oligo #42 [SEQ ID NO:56]

15 **Oligo #37** [SEQ ID NO:51]

Oligo #38 [SEQ ID NO:52]

Oligo #33 [SEQ ID NO:47]

Oligo #34 [SEQ ID NO:48]

20

Oligo #43 [SEQ ID NO:57]

Oligo #44 [SEQ ID NO:58]

The assembled oligonucleotides create NsiI and EcoRI
25 restriction ends and the DNA sequence that encodes amino acids 72-105 of (15-125)hIL-3 with the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A and 105Q. The codons encoding amino acids 72-105 of (15-125)hIL-3 are those found in the hIL-3
30 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was
35 isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of

the oligonucleotides. The plasmid, pMON13355, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

5 **Peptide #9**
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 10 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:73]

DNA sequence #17 [SEQ ID NO:113] codes for the foregoing pMON13355 polypeptide.
 30

EXAMPLE 18

Construction of pMON13359

Plasmid pMON5988 DNA was digested with restriction
 35 enzymes EcoRI and HindIII, and the resulting 4225 base pair EcoRI,HindIII fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, g10L
 40 ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-105 of (15-125)hIL-3. The 4225 base pair EcoRI,HindIII restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides.

45

Oligo #17 [SEQ ID NO:31]

Oligo #18 [SEQ ID NO:32]

5 The ligation reaction was used to transform E. coli K-
12 strain JM101. Transformant bacteria were selected
on ampicillin-containing plates. Plasmid DNA was
isolated from a colony grown in LB broth, and the size
of the inserted fragment was determined by restriction
10 analysis employing restriction enzymes NcoI and HindIII
in double digest. In the resulting plasmid the 64
bases between the EcoRI and HindIII restriction sites
in the (15-125)hIL-3 gene are replaced with 20 bases
from the above mentioned oligonucleotides. This linker
15 also contains an NdeI recognition sequence.

EXAMPLE 19

Construction of pMON13352

20 The 4225 base pair EcoRI,HindIII restriction fragment
from pMON13359 was ligated with the following pairs of
annealed complementary oligonucleotides:

Oligo #45 [SEQ ID NO:59]

25 **Oligo #46** [SEQ ID NO:60]

Oligo #49 [SEQ ID NO:63]

Oligo #50 [SEQ ID NO:64]

30 The assembled oligonucleotides create EcoRI and HindIII
restriction ends and the DNA sequence that encodes
amino acids 106-125 of (15-125)hIL-3 with the following
amino acid substitutions: 109E, 116V, 120Q and 123E.
The codons encoding amino acids 106-125 of (15-125)hIL-
35 3 are those found in the hIL-3 cDNA sequence except at
those positions where amino acid substitutions were

made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13352, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #10**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

15 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

20 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

25 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]

DNA sequence #18 [SEQ ID NO:114] codes for the foregoing pMON13352 polypeptide.

35

EXAMPLE 20

Construction of pMON13354

The 4225 base pair EcoRI, HindIII restriction fragment from pMON13359 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #45 [SEQ ID NO:59]

Oligo #46 [SEQ ID NO:60]

45

EXAMPLE 21Construction of pMON13360

5 Plasmid pMON13352 DNA was digested with restriction
enzymes NsiI and EcoRI, resulting in a 4178 base pair
NsiI,EcoRI fragment. The genetic elements derived from
pMON13352 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage f1 origin of replication
10 as the transcription terminator, pAraBAD promoter, g10L
ribosome binding site, lamB secretion leader and the
bases encoding amino acids 15-71 and 106-125 of (15-
125)hIL-3. Plasmid pMON13350 DNA was digested with
NsiI and EcoRI. The resulting 111 base pair NsiI,
15 EcoRI fragment encodes amino acids 72-105 of (15-
125)hIL-3. The eluted restriction fragments were
concentrated and desalted using Centricon 30
concentrators. The restriction fragments were ligated,
and the ligation reaction mixture was used to transform
20 E. coli K-12 strain JM101. Transformant bacteria were
selected on ampicillin-containing plates. Plasmid DNA
was isolated and analyzed by restriction analysis.
Clones containing the correct insert lost a XmnI site
as compared with pMON13352. Positive clones were
25 identified by the loss of a 615 base pair XmnI
fragment. The DNA was sequenced to confirm the correct
insert. The resulting (15-125)hIL-3 variant has the
following amino acid substitutions: 73G, 76A, 79R,
82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and
30 123E. The codons encoding amino acids 72-125 of (15-
125)hIL-3 are those found in the hIL-3 cDNA sequence
except at those positions where amino acid
substitutions were made. The plasmid, pMON13360,
encodes the (15-125)hIL-3 variant with the following
35 amino acid sequence:

Peptide #12

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

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Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
5 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
10 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ. NO:76]

DNA sequence #23 [SEQ ID NO:119] encodes the
foregoing pMON13360 polypeptide.
25

EXAMPLE 22

Construction of pMON13361

Plasmid pMON13352 DNA was digested with restriction
30 enzymes NsiI and EcoRI, resulting in a 4178 base pair
NsiI,EcoRI fragment. The genetic elements derived from
pMON13352 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage f1 origin of replication
as the transcription terminator, pAraBAD promoter, g10L
35 ribosome binding site, lamB secretion leader and the
bases encoding amino acids 15-71 and 106-125 of (15-
125)hIL-3. Plasmid pMON13355 DNA was digested with
NsiI and EcoRI. The resulting 111 base pair NsiI,
EcoRI fragment encodes amino acids 72-105 of (15-
40 125)hIL-3. The restriction fragments were ligated, and
the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
selected on ampicillin-containing plates. Clones
containing the correct insert contained an additional
45 RsaI site which results in a 1200 base pairs RsaI

220

fragment. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E.

5 The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13361, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #13**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

15 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

20 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:77]

DNA sequence #24 [SEQ ID NO:120] codes for the foregoing pMON13361 polypeptide.

35

EXAMPLE 23

Construction of pMON13362

Plasmid pMON13354 DNA was digested with restriction
40 enzymes NsiI and EcoRI, resulting in a 4178 base pair
NsiI,EcoRI fragment. The genetic elements derived from
pMON13354 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage fl origin of replication
as the transcription terminator, pAraBAD promoter, g10L
45 ribosome binding site, lamB secretion leader and the

bases encoding amino acids 15-71 and 106-125 of (15-125)hIL-3. Plasmid pMON13355 DNA was digested with NsiI and EcoRI. The resulting 111 base pair NsiI, EcoRI fragment encodes amino acids 72-105 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Clones containing the correct insert contained an additional RsaI site which results in a 1200 base pairs RsaI fragment. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13362, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #14

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:78]

DNA sequence #25 [SEQ ID NO:121] codes for the

foregoing pMON13362 polypeptide.

EXAMPLE 24

5 Construction of pMON13363

Plasmid pMON13344 DNA was digested with restriction enzymes NsiI and EcoRV, resulting in a 4218 base pair NsiI,EcoRV fragment. The genetic elements derived from pMON13344 are the beta-lactamase gene (AMP), pBR327
10 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid pMON13348 DNA was digested with
15 NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV fragment encodes amino acids 47-71 of (15-125)hIL-3. The restriction fragments were ligated with T4 ligase, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were
20 selected on ampicillin-containing plates. Clones containing the correct insert contained an additional DdeI site which results in DdeI restriction fragments of 806 and 167 base pairs compared to 973 base pairs in pMON13344. The DNA was sequenced to confirm the
25 correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence
30 except at those positions where amino acid substitutions were made. The plasmid, pMON13363, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #15

35 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:79]
20 **DNA sequence #20** [SEQ ID NO:116] codes for the
foregoing pMON13363 polypeptide.

EXAMPLE 25

25 Construction of pMON13364
Plasmid pMON13345 DNA was digested with restriction
enzymes NsiI and EcoRV, resulting in a 4218 base pair
NsiI,EcoRV fragment. The genetic elements derived from
pMON13345 are the beta-lactamase gene (AMP), pBR327
30 origin of replication, phage fl origin of replication
as the transcription terminator, pAraBAD promoter, g10L
ribosome binding site, lamB secretion leader and the
bases encoding amino acids 15-46 and 72-125 of (15-
125)hIL-3. Plasmid pMON13349 DNA was digested with
35 NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV
fragment encodes amino acids 47-71 of (15-125)hIL-3.
The restriction fragments were ligated, and the
ligation reaction mixture was used to transform E. coli
K-12 strain JM101. Transformant bacteria were selected
40 on ampicillin-containing plates. Clones containing the
correct insert contained an additional DdeI site which
results in DdeI restriction fragments of 806 and 167
base pairs compared to 973 base pairs in pMON13344.

40 Plasmid pMON13346 DNA was digested with restriction
enzymes NsiI and EcoRV, resulting in a 4218 base pair
NsiI,EcoRV fragment. The genetic elements derived from
pMON13346 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage fl origin of replication
45 as the transcription terminator, pAraBAD promoter, g10L

ribosome binding site, lamB secretion leader and the
bases encoding amino acids 15-46 and 72-125 of (15-
125)hIL-3. Plasmid pMON13347 DNA was digested with
NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV
5 fragment encodes amino acids 47-71 of (15-125)hIL-3.
The restriction fragments were ligated, and the
ligation reaction mixture was used to transform E. coli
K-12 strain JM101. Transformant bacteria were selected
on ampicillin-containing plates. Clones containing the
10 correct insert contained an additional DdeI site which
results in DdeI restriction fragments of 806 and 167
base pairs compared to 973 base pairs in pMON13344.
The DNA was sequenced to confirm the correct insert.
The resulting (15-125)hIL-3 variant has the following
15 amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S,
45M, 51R, 55L, 59L, 62V, 67N and 69E. The codons
encoding amino acids 15-71 of (15-125)hIL-3 are those
found in the hIL-3 cDNA sequence except at those
positions where amino acid substitutions were made.
20 The plasmid, pMON13365, encodes the (15-125)hIL-3
variant with the following amino acid sequence:

Peptide #17

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
25 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
30 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
35 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
40 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:81]

45 **DNA sequence #22** [SEQ ID NO:118] codes for the

foreging pMON13365 polypeptide.

EXAMPLE 27

5 Construction of pMON13298

Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair NsiI,HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, preA promoter, gl0L ribosome binding site, and the bases encoding amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13360 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13298, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #18

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

35 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

40

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Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82]
 15 **DNA sequence #29** [SEQ ID NO:125] codes for the
 foregoing pMON13298 polypeptide.

EXAMPLE 28

20 Construction of pMON13299
 Plasmid pMON5978 DNA was digested with restriction
 enzymes NsiI and HindIII, resulting in a 3789 base pair
 NsiI, HindIII fragment. The genetic elements derived
 from pMON5978 are the beta-lactamase gene (AMP), pBR327
 25 origin of replication, phage fl origin of replication
 as the transcription terminator, precA promoter, gl0L
 ribosome binding site and the bases encoding amino
 acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA
 was digested with NsiI and HindIII, the resulting 175
 30 base pair NsiI, HindIII fragment encodes amino acids
 72-125 of (15-125)hIL-3. The restriction fragments
 were ligated, and the ligation reaction mixture was
 used to transform E. coli K-12 strain JM101.
 Transformant bacteria were selected on ampicillin-
 35 containing plates. Plasmid DNA was isolated, analyzed
 by restriction analysis, and sequenced to confirm the
 correct insert. The resulting (15-125)hIL-3 variant
 has the following amino acid substitutions: 73G, 76A,
 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q
 40 and 123E. The codons encoding amino acids 72-125 of
 (15-125)hIL-3 are those found in the hIL-3 cDNA

sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13299, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

5 **Peptide #19**
 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 10 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83]

DNA sequence #30 [SEQ ID NO:126] codes for the foregoing pMON13299 polypeptide.

30

EXAMPLE 29

Construction of pMON13300

35 Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair NsiI,HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication
 40 as the transcription terminator, precA promoter, g10L ribosome binding site, and the bases encoding amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids
 45 72-125 of (15-125)hIL-3. The restriction fragments

enzymes NcoI and NsiI, resulting in a 3794 base pair
NcoI,NsiI fragment. The genetic elements derived from
pMON5978 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage fl origin of replication
5 as the transcription terminator, precA promoter, g10L
ribosome binding site and the bases encoding amino
acids 72-125 of (15-125)hIL-3. Plasmid pMON13363 DNA
was digested with NcoI and NsiI. The resulting 170
base pair NcoI, NsiI fragment encodes amino acids 15-71
10 of (15-125)hIL-3. The restriction fragments were
ligated, and the ligation reaction mixture was used to
transform E. coli K-12 strain JM101. Transformant
bacteria were selected on ampicillin-containing plates.
Plasmid DNA was isolated, analyzed by restriction
15 analysis, and sequenced to confirm the correct insert.
The resulting (15-125)hIL-3 variant has the following
amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A,
45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons
encoding amino acids 15-71 of (15-125)hIL-3 are those
20 found in the hIL-3 cDNA sequence except at those
positions where amino acid substitutions were made.
The plasmid, pMON13301, encodes the (15-125)hIL-3
variant with the following amino acid sequence:

Peptide #21

25 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
40 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
45

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

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Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 5 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 15 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:86]

DNA sequence #27 [SEQ ID NO:123] codes for the
 20 foregoing pMON13302 polypeptide.

EXAMPLE 32

Construction of pMON13303
 Plasmid pMON5978 DNA was digested with restriction
 25 enzymes NcoI and NsiI, resulting in a 3794 base pair
 NcoI,NsiI fragment. The genetic elements derived from
 pMON5978 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage fl origin of replication
 as the transcription terminator, precA promoter, gl0L
 30 ribosome binding site, and the bases encoding amino
 acids 72-125 of (15-125)hIL-3. Plasmid pMON13365 DNA
 was digested with NcoI and NsiI. The resulting 170
 base pair NcoI, NsiI fragment encodes amino acids 15-71
 of (15-125)hIL-3. The restriction fragments were
 35 ligated, and the ligation reaction mixture was used to
 transform E. coli K-12 strain JM101. Transformant
 bacteria were selected on ampicillin-containing plates.
 Plasmid DNA was isolated, analyzed by restriction
 analysis, and sequenced to confirm the correct insert.
 40 The resulting (15-125)hIL-3 variant has the following
 amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S,
 45M, 51R, 55L, 59L, 62V, 67N and 69E. The codons

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encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13303, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #23

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

10 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

15 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

20 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

25 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:87]

30 **DNA sequence #28** [SEQ ID NO:124] codes for the foregoing pMON13303 polypeptide.

EXAMPLE 33

35 Construction of pMON13287

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13363 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13360 DNA was digested with

45

30 Peptide #25
Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
35 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
40

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]

DNA sequence #4 [SEQ ID NO:100] codes for the
 foregoing pMON13288 polypeptide.
 15

EXAMPLE 35

Construction of pMON13289
 Plasmid pMON2341 DNA was digested with restriction
 20 enzymes NcoI and HindIII, resulting in a 3619 base pair
 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage fl origin of replication
 as the transcription terminator, precA promoter and
 25 gl0L ribosome binding site. Plasmid pMON13365 DNA was
 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 (15-125)hIL-3. Plasmid pMON13360 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 30 HindIII fragment encodes amino acids 72-125 of (15-
 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 35 was isolated, analyzed by restriction analysis, and
 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R,
 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S,
 40 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons

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encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13289, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #26

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

10 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
15 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]

30 **DNA sequence #7** [SEQ ID NO:103] codes for the foregoing pMON13289 polypeptide.

35 EXAMPLE 36

Construction of pMON13290

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived
40 from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13363 DNA was digested with NcoI and NsiI. The resulting 170 base
45 pair NcoI, NsiI fragment encodes amino acids 15-71 of

EXAMPLE 37

Construction of pMON13292

Plasmid pMON2341 DNA was digested with restriction
 5 enzymes NcoI and HindIII, resulting in a 3619 base pair
 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage f1 origin of replication
 as the transcription terminator, precA promoter and
 10 gl0L ribosome binding site. Plasmid pMON13365 DNA was
 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 (15-125)hIL-3. Plasmid pMON13361 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 15 HindIII fragment encodes amino acids 72-125 of (15-
 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 20 was isolated, analyzed by restriction analysis, and
 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R,
 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S,
 25 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons
 encoding amino acids 15-125 of (15-125)hIL-3 are those
 found in the hIL-3 cDNA sequence except at those
 positions where amino acid substitutions were made.
 The plasmid, pMON13292, encodes the (15-125)hIL-3
 30 variant with the following amino acid sequence:

Peptide #28

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 35 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]
 15 **DNA sequence #8** [SEQ ID NO:104] codes for the
 foregoing pMON13292 polypeptide.

EXAMPLE 38

20 Construction of pMON13294

Plasmid pMON2341 DNA was digested with restriction
 enzymes NcoI and HindIII, resulting in a 3619 base pair
 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 25 origin of replication, phage f1 origin of replication
 as the transcription terminator, precA promoter and
 g10L ribosome binding site. Plasmid pMON13364 DNA was
 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 30 (15-125)hIL-3. Plasmid pMON13362 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 HindIII fragment encodes amino acids 72-125 of (15-
 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform E.
 35 coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 was isolated, analyzed by restriction analysis, and
 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 40 substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R,
 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S,

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98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made.

- 5 The plasmid, pMON13294, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #29

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 10 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:93]

30

DNA sequence #6 [SEQ ID NO:102] codes for the foregoing pMON13294 polypeptide.

35

EXAMPLE 39

Construction of pMON13295

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair
 40 NcoI,HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13365 DNA was
 45 digested with NcoI and NsiI. The resulting 170 base

pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13295, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #30

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:94]

DNA sequence #9 [SEQ ID NO:105] codes for the foregoing pMON13295 polypeptide.

EXAMPLE 40

Construction of pMON13312

5 Plasmid pMON2341 DNA was digested with restriction
enzymes NcoI and HindIII, resulting in a 3619 base pair
NcoI,HindIII fragment. The genetic elements derived
from pMON2341 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage fl origin of replication
10 as the transcription terminator, precA promoter and
g10L ribosome binding site. Plasmid pMON13364 DNA was
digested with NcoI and NsiI. The resulting 170 base
pair NcoI, NsiI fragment encodes amino acids 15-71 of
(15-125)hIL-3. Plasmid pMON13361 DNA was digested with
15 NsiI and HindIII. The resulting 175 base pair NsiI,
HindIII fragment encodes amino acids 72-125 of (15-
125)hIL-3. The restriction fragments were ligated, and
the ligation reaction mixture was used to transform E.
coli K-12 strain JM101. Transformant bacteria were
20 selected on ampicillin-containing plates. Plasmid DNA
was isolated, analyzed by restriction analysis, and
sequenced to confirm the correct insert. The resulting
(15-125)hIL-3 variant has the following amino acid
substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R,
25 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S,
98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons
encoding amino acids 15-125 of (15-125)hIL-3 are those
found in the hIL-3 cDNA sequence except at those
positions where amino acid substitutions were made.
30 The plasmid, pMON13312, encodes the (15-125)hIL-3
variant with the following amino acid sequence:

Peptide #31

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

35

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 5 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:95]

DNA sequence #5 [SEQ ID NO:101] codes for the
 foregoing pMON13312 polypeptide.

20

EXAMPLE 41

Construction of pMON13313

Plasmid pMON2341 DNA was digested with restriction
 enzymes NcoI and HindIII, resulting in a 3619 base pair
 25 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage fl origin of replication
 as the transcription terminator, precA promoter and
 g10L ribosome binding site. Plasmid pMON13363 DNA was
 30 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 (15-125)hIL-3. Plasmid pMON13362 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 HindIII fragment encodes amino acids 72-125 of (15-
 35 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform E.
coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 was isolated, analyzed by restriction analysis, and
 40 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R,

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55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13313, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #32

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
15 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
30 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:96]

DNA sequence #3 [SEQ ID NO:99] codes for the foregoing pMON13313 polypeptide.

35 EXAMPLE 42

Construction of pMON5987

Plasmid pMON6458 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3940 base pair
40 NcoI,HindIII fragment. The genetic elements derived from pMON6458 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site and lamB secretion leader.
45 Plasmid pMON5978 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes

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amino acids 15-71 of (15-125)hIL-3. Plasmid pMON5976
DNA was digested with NsiI and HindIII. The resulting
175 base pair NsiI,HindIII fragment encodes amino acids
72-125 of (15-125)hIL-3. The restriction fragments
5 were ligated, and the ligation reaction mixture was
used to transform E. coli K-12 strain JM101.
Transformant bacteria were selected on ampicillin-
containing plates. Plasmid DNA was isolated and
screened for the restriction sites EcoRV and NheI and
10 DNA sequenced to confirm the correct insert.

EXAMPLE 43

Construction of pMON5988

15 The plasmid DNA of pMON5987 was digested with NheI
and EcoRI, resulting in a 3903 base pair NheI, EcoRI
fragment. The 3903 base pair NheI, EcoRI fragment was
ligated to 1.0 picomoles of the following annealed
oligonucleotides:

20

5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA-
3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTT-

GGACGGTGACTGGAATG-3' [SEQ ID NO:131]

25 CCTGCCACTGACCTTACAATT-5' [SEQ ID NO:132]

The ligation reaction mixture was used to
transform E. coli K-12 strain JM101 and transformant
bacteria were selected on ampicillin-containing plates.
30 Plasmid DNA was isolated and sequenced to confirm
positive clones. This plasmid was constructed to
change alanine 101 to aspartic acid in the hIL-3 gene
(15-125). This plasmid was designated pMON5988.

35

EXAMPLE 44

Construction of pMON5853 (Fig 6) which encodes [Met-(15-133)hIL-3(Arg¹²⁹)]

Plasmid DNA of pMON5847 (Example 2) was treated with NcoI. The restriction enzyme was inactivated by
5 heat treatment (65°C for 10 minutes). The DNA was then treated with large fragment of DNA polymerase I (Klenow) in the presence of all four nucleotide precursors. This produces DNA termini with non-overlapping ends. After 5 minutes at 37°C, the
10 polymerase was inactivated by heat treatment at 65°C for 10 minutes. The DNA was then treated with HpaI, an enzyme which produces non-overlapping termini. The DNA was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101
15 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis. A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction
20 of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:133]

M N C S N [SEQ ID NO:134]

25

The lower line contains the one letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and
30 asparagine.

When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

35 The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg¹²⁹) which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 5 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg
 10 Leu Ala Ile Phe [SEQ ID NO:135]

EXAMPLE 45

Construction of pMON5873 which encodes [Met-(1-133)hIL-
 15 3]

The gene obtained from British Biotechnology, Ltd. specified arginine at codon position 129. The amino acid specified in the native hIL-3 cDNA is serine. To produce a protein with the native sequence at this
 20 position, the portion of the coding sequence between the EcoRI site at codons 106 and 107 and the NheI site at codons 129 and 130 was replaced. Plasmid DNA of pMON5854 (Example 3) and pMON5853 (Example 44) were treated with EcoRI and NheI. The larger fragments of
 25 each were gel purified. These were ligated to a pair of an annealed oligonucleotides with the following sequences:

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAACC-
 30 3'-GGCAGCATTTGACTGGAAGATAGACTTTTGG-

TTGGAGAACGCGCAGGCTCAACAGACCACTCTGTCG-3' [SEQ ID NO:
 136]
 AACCTCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATC-5' [SEQ ID
 35 NO:137]

The ligation reaction mixtures were used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and grown. Plasmid DNA was isolated and screened for the presence of a new StyI recognition site present in the synthetic DNA and not in pMON5854 and pMON5853. The nucleotide sequence of the gene in the region between EcoRI and NheI was determined and found to be that of the synthetic oligonucleotides. The new plasmids were designated pMON5873 encoding [Met-(1-133)hIL-3] and pMON5872 encoding [Met-(15-133)hIL-3].

The plasmid, pMON5873, encodes Met-(1-133)hIL-3 which has the following amino acid sequence:
 Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
 Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser
 Leu Ala Ile Phe [SEQ ID NO:128]

25

EXAMPLE 46

Construction of pMON6458

Plasmid pMON6525 was digested with restriction enzymes HindIII and SalI and the resulting 3172 base pair fragment was isolated from a 1% agarose gel by interception onto DEAE membrane. The genetic elements derived from pMON6525 are the beta-lactamase gene (AMP), pBR327 origin of replication, and phage f1 origin of replication as the transcription terminator. (The genetic elements derived from plasmid pMON6525 are

35

identical to those in plasmid pMON2341 which could also be used to construct pMON6458.) Plasmid pMON6457 was digested with restriction enzymes HindIII and SalI and the resulting 1117 base pair fragment was isolated by
5 PAGE and crush and soak elution. The genetic elements derived from pMON6457 are the pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the (15-125) hIL-3 gene. The restriction fragments were ligated and the ligation reaction mixture was used to
10 transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in
15 double digest. Clones containing the hIL-3 gene (encoding amino acids 15-125) contained a 345 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6458. This plasmid was constructed to eliminate an EcoRI restriction site outside the hIL-3
20 gene coding region in plasmid pMON6457.

EXAMPLE 47

Construction of pMON5976 which encodes [Met-(15-
25 125)hIL-3(Ala¹⁰¹)]

The plasmid DNA of pMON5941 isolated from the dam-
E. coli strain GM48 was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six oligonucleotides encoding amino acids 20-70 of hIL-3
30 (FIG. 2). This synthetic fragment encodes three unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in Figure 2.

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant
35 colonies. Plasmid DNA was isolated and the inserted fragment was determined to have both an EcoRV and NheI

site. The nucleotide sequence of the region between
 ClaI and NsiI was determined and found to be that of
 the synthetic oligonucleotides. At codons 86-87 of a
 nucleotide sequence coding for (15-125)hIL-3, an NheI
 5 site was introduced. The plasmid with this alteration
 was designated pMON5941. This plasmid encodes Met-(15-
 125)hIL-3 which is altered at position 101 by
 replacement of aspartate by alanine.

10 Plasmid pMON5976 encodes Met-(15-125)hIL-3(Ala¹⁰¹)
 which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 15 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Ala Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 20 Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:138]

EXAMPLE 48

Construction of pMON5917 which encodes [Met-(15-88)hIL-
 25 3]

The plasmid DNA of pMON5853 was cleaved with NsiI
 and HindIII and ligated to an annealed pair of
 oligonucleotides encoding (70-88)hIL-3 with a new NheI
 endonuclease restriction site at codons 86-87. The
 30 sequence of these oligonucleotides is shown in
 Example 18.

The ligation mixture was used to transform
 competent E. coli JM101 cells, and ampicillin resistant
 colonies were picked. Plasmid DNA isolated from
 35 individual colonies was screened for the presence of
 the new NheI restriction site. The nucleotide sequence

of the substituted portion was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5917 encoding Met-(15-88)hIL-3 containing a new NheI site at codons 86-87.

5 Plasmid pMON5917 encodes Met-(15-88)hIL-3 which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
10 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala [SEQ ID NO:
139]

15

EXAMPLE 49

Construction of pmon5941 which encodes [Met-(15-125)hIL-3 Ala¹⁰¹]

The plasmid DNA of pMON5917 was cleaved with NheI
20 and HindIII and ligated to two annealed pairs of
oligonucleotides which encode amino acids 86-106 and
107-125 of hIL-3. The sequences of these
oligonucleotides is shown below.
NheI to EcoRI

25 5'-CTAGCCACGGCCGACCCACGCGACATCCAATCCATATCAAGGCTG-
3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTTCCGAC-

GTGACTGGAATG-3' [SEQ ID NO:140]

CACTGACCTTACTTAA-5' [SEQ ID NO:141]

30

EcoRI to HindIII

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA-
3'-GGCAGCATTTGACTGGAAGATAGACTTTTGAACCTCTTGCGCGT-

35 GGCTCAACAGTAATA-3' [SEQ ID NO:142]

CCGAGTTGTCATTATTCGA-5' [SEQ ID NO:143]

The ligation mixture was used to transform competent E. coli JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be larger by restriction analysis with NcoI and HindIII. The Asp to Ala 101 change is encoded on the NheI to EcoRI fragment. The nucleotide sequence of the portion of the coding region between the NheI and HindIII sites was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5941.

The plasmid, pMON5941, encodes Met-(15-125)hIL-3(Ala¹⁰¹) and contains a new NheI restriction site.

15

EXAMPLE 50

Construction of pMON6455

Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and phage fl origin of replication as the transcription terminator. The following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, gl0L ribosome binding site and phage fl origin of replication as the transcription terminator, derived from plasmid pMON5905 are identical to these in plasmid pMON5594 which could also be used to construct pMON6455. The AraBAD promoter is identical to that described in pMON6235. The lamB signal peptide sequence used in pMON6455 is that shown in Figure 8 fused to hIL-3 (15-125) at the NcoI site. Plasmid pMON5887 was digested with restriction enzymes HindIII and NcoI, resulting in a

30

35

384 base pair NcoI, HindIII fragment. The restriction fragments were ligated, and the ligation reaction mixture was used to transform into E. coli K-12 strain JM101. Transformant bacteria were selected on
5 ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Positive clones
10 containing the hIL-3 gene (encoding amino acids 1-125) contained a 384 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6455.

EXAMPLE 51

Construction of pMON6456

15 Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the
20 transcription terminator, pAraBAD promoter, gl0L ribosome binding site and the lamB secretion leader. Plasmid pMON5871 was digested with restriction enzymes HindIII and NcoI, resulting in a 330 base pair NcoI, HindIII fragment. The genetic element derived from
25 pMON5871 encompassed the bases encoding the (1-107) hIL-3 gene. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA
30 was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 1-107) contained a 330 base pair NcoI, HindIII
35 restriction fragment. This construct was designated pMON6456.

EXAMPLE 52

5 Construction of pMON6457

Plasmid pMON6455 DNA grown in E. coli strain GM48 (dam-) was digested with restriction enzymes NcoI and ClaI, resulting in a 4263 base pair NcoI, ClaI fragment. The restriction fragment was ligated to 1.0
10 picomoles of annealed oligonucleotides with the following sequence coding for Met Ala 14-20 hIL-3:

5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:151]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:152]

15

The resulting DNA was transformed into E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was
20 determined by restriction analysis employing restriction enzymes XbaI and EcoRI in double digest. Positive clones containing the hIL-3 gene (encoding aa 15-125 of hIL-3) contained a 433 base pair XbaI, EcoRI restriction fragment. This construct was designated
25 pMON6457. This plasmid was constructed to delete the first 14 amino acids of hIL-3. The coding sequence of the resulting gene begins as follows:

5' ATG GCT AAC TGC... 3' [SEQ ID NO:153]

30 Met Ala Asn Cys... [SEQ ID NO:154]

15

The first two amino acids (Methionine, Alanine) create an NcoI restriction site and a signal peptidase
35 cleavage site between the lamB signal peptide and (15-125) hIL-3. Plasmid pMON6457 encodes (15-125) hIL-3

which has the amino acid sequence designated SEQ ID NO:65.

5 EXAMPLE 53

Construction of pMON6235

One of the DNA fragments used to create this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the following
 10 oligonucleotides, Oligo #51 [SEQ ID NO:155] and Oligo #52 [SEQ ID NO:156], were used as primers in this procedure. The template for the PCR reaction was E. coli strain W3110 chromosomal DNA, prepared as described in Maniatis (1982). The oligonucleotide
 15 primers were designed to amplify the AraBAD promoter (Greenfield et al., 1978). The resulting DNA product was digested with the restriction enzymes SacII and BglII. The reaction mixture was purified as described previously. Plasmid, pMON5594, DNA was digested with
 20 SacII and BglII, resulting in a 4416 base pair SacII,BglII restriction fragment which contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, G10L ribosome binding site, phage fl origin of replication as the
 25 transcription terminator and the chloramphenicol acetyl transferase (cat) gene. The 4416 base pair SacII,BglII restriction fragment from pMON5594 was ligated to the PCR-generated SacII, BglII DNA fragment. The ligation mixture was used to transform E. coli K-112 strain
 30 JM101. Positive clones contained a 323 base pair SacII,BglII fragment and were DNA sequenced to confirm that the SacII,BglII fragment was the AraBAD promoter. This construct was designated pMON6235.

35

EXAMPLE 54

Construction of pMON5647

Plasmid pMON5585 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3273 base pair NcoI,HindIII fragment. The genetic elements derived from pMON5585 are the pBR327 origin of replication, precA promoter, gl0L ribosome binding protein, bovine somatotropin gene (bST), beta-lactamase gene (AMP) and T7 transcription terminator. Plasmid pMON3267 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was digested with NcoI and HindIII enzymes resulting in a 580 base pair NcoI,HindIII fragment which contains the porcine somatotropin (pST) gene. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert.

EXAMPLE 55

Construction of pMON710

Plasmid pMON709 consists of a 1614 base pair AvaI,EcoRI fragment of transposon TN7, containing the streptomycin adenylyltransferase gene (Fling et al., 1985) and a pUC9 linker (XmaI,HindIII) cloned between the HindIII and EcoRI sites of pUC19. The streptomycin adenylyltransferase gene confers resistance to streptomycin and spectinomycin. Plasmid pMON709 was mutagenized by oligonucleotide site-directed mutagenesis (methods described in Zoller and Smith, 1982) to introduce an EcoRV site at the 3' end of the streptomycin adenylyltransferase gene. The oligonucleotide, **Oligo # 53** [SEQ ID NO:157], was used

in this procedure to introduce the EcoRV site. The resulting plasmid was designated pMON710.

5 EXAMPLE 56

Construction of pMON5723

Plasmid pMON5647 DNA was digested with restriction enzymes DraI and SspI resulting in a 2916 base pair DraI, SspI fragment. The genetic elements derived from pMON5647 are the pBR327 origin of replication, precA promoter, gl0L ribosome binding protein, porcine somatotropin gene (pST) and T7 transcription terminator (Dunn and Strudier, 1983). Plasmid pMON710 DNA was digested with restriction enzymes HincII and EcoRV resulting in 940 base pair HincII,EcoRV fragment containing the streptomycin adenylyltransferase gene which confers resistance to streptomycin and spectinomycin. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. The DraI, SspI, HincII and EcoRV restriction sites are lost as a result of the cloning. Transformant bacteria were selected on spectinomycin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert.

EXAMPLE 57

Construction of pMON13361

Plasmid pMON13288 was mutagenized by oligonucleotide site-directed mutagenesis (method described in Kunkel, 1985) to eliminate a NsiI site in the (15-125) hIL-3 variant coding region. Codon 70 of (15-125) hIL-3, encoding asparagine, was converted from AAT to AAC destroying the NsiI recognition site. The oligonucleotide, **Oligo # 54** [SEQ ID NO:158], was used

in this procedure to eliminate the NsiI site. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis to confirm the loss of the NsiI site and sequenced to confirm the sequence of the (15-125) hIL-3 variant gene. The plasmid, pMON13361, encodes the (15-125) hIL-3 variant with the amino acid sequence of PEPTIDE #25 [SEQ ID NO:89].

DNA sequence # 32 [SEQ ID NO:160] codes for the foregoing pMON13361 polypeptide.

EXAMPLE 58

Construction of pMON14058

Plasmid pMON13361 was mutagenized by oligonucleotide site-directed mutagenesis (method described by Taylor et al., 1985 using a kit from Amersham, Arlington Heights, Ill.) to eliminate a EcoRV site in the (15-125) hIL-3 variant coding region. Codon 46 and 47 of (15-125) hIL-3, encoding asparagine and isoleucine, were converted from GAT to GAC and ATC to ATT respectively, destroying the EcoRV recognition site. The oligonucleotide, **Oligo # 55** [SEQ ID NO:159], was used in this procedure to eliminate the EcoRV site.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis to confirm the loss of the EcoRV site and sequenced to confirm the sequence of the (15-125) hIL-3 variant gene. The plasmid, pMON14058, encodes the (15-125) hIL-3 variant with the amino acid sequence of **PEPTIDE #25** [SEQ ID NO:89].

DNA sequence # 33 [SEQ ID NO:161] codes for the foregoing pMON14058 polypeptide.

EXAMPLE 59

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acids 47-125 of (15-125) hIL-3 with the following amino acid substitution, 50D. The 3669 base pair NcoI,EcoRV restriction fragment from pMON13252 was ligated to the following annealed complementary oligonucleotides.

5

Oligo #165 [SEQ ID NO:162]

Oligo #166 [SEQ ID NO:163]

Oligo #167 [SEQ ID NO:164]

10 Oligo #168 [SEQ ID NO:165]

Oligo #169 [SEQ ID NO:166]

Oligo #170 [SEQ ID NO:167]

15 When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125) hIL-3 with the following amino acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3
20 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13285, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

25 Peptide #A3 [SEQ ID NO:258]

DNA sequence #A3 pMON13285 42D, 45M 46S, 50D

30 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT
CTATCCTGAT GGACAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
35 CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
40 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC

TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:398]

5 EXAMPLE 61

Construction of pMON13286

Plasmid pMON5978 DNA was digested with restriction
10 enzymes NcoI and EcoRV and the resulting 3865 base pair
NcoI,EcoRV fragment contains the following genetic
elements; beta-lactamase gene (AMP), pBR327 origin of
replication, phage f1 origin of replication as the
transcription terminator, precA promoter, gl0L ribosome
15 binding site and the bases encoding amino acids 47-125
of (15-125) hIL-3. The 3865 base pair NcoI,EcoRV
restriction fragment from pMON5978 was ligated to the
following annealed complementary oligonucleotides.

```

20  Oligo #165      [SEQ ID NO:162]
    Oligo #166      [SEQ ID NO:163]
    Oligo #167      [SEQ ID NO:164]
    Oligo #168      [SEQ ID NO:165]

```

25 Oligo #169 [SEQ ID NO:166]
 Oligo #170 [SEQ ID NO:167]

When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that
30 encodes amino acids 15-46 of (15-125) hIL-3 with the following amino acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were
35 made. The plasmid, pMON13286, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

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Peptide #A4 [SEQ ID NO:259]

DNA sequence #A4 pMON13286 42D, 45M, 46S

5 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT
10 CTATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
15 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:399]

20

EXAMPLE 62

Construction of pMON13325

25 The 3704 base pair EcoRI, HindIII DNA fragment from
plasmid pMON13286 is ligated to the 64 base pair EcoRI,
HindIII DNA fragment from plasmid pMON13215. The
following genetic elements are derived from pMON13286;
beta-lactamase gene (AMP), pBR327 origin of
30 replication, phage F1 origin of replication as the
transcription terminator, precA promoter, g10L ribosome
binding site and the bases encoding amino acids 15-105
of the (15-125) hIL-3 gene with the following changes,
42D, 45M, and 46S. The bases encoding amino acids
35 106-125 of the (15-125) gene with the following change,
116W, are derived from pMON13215. The resulting
plasmid, pMON13325, encodes the (15-125) hIL-3 variant
with the following amino acid sequence:

40 Peptide # A5 [SEQ ID NO:261]

265

ligated to the following annealed complementary oligonucleotides.

Oligo #15(A) [SEQ ID NO:168]

5

Oligo #16(A) [SEQ ID NO:169]

In the resulting plasmid the 111 bases between the NsiI and EcoRI restriction sites in the (15-125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also creates a NdeI recognition sequence.

10

EXAMPLE 65

15

Construction of pMON13330

The 3846 base pair PstI, EcoRI DNA fragment from plasmid pMON13332 is ligated to the 118 base pair PstI, EcoRI DNA fragment from plasmid pMON13305. The following genetic elements are derived from pMON13332; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, gl0L ribosome binding site and the bases encoding amino acids 15-69 and 106-125 of the (15-125) hIL-3 gene with the following change, 42D, 45M, 46S, 50D and 116W. The bases encoding amino acids 70-105 of the (15-125) gene with the following change, 95R, 98I and 100R are derived from pMON13305. The resulting plasmid, pMON13330, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

25

30

Peptide # A7 [SEQ ID NO:263]

35

EXAMPLE 66

Construction of pMON13329

The 3846 base pair PstI, EcoRI DNA fragment from
 5 plasmid pMON13332 is ligated to the 118 base pair
 PstI, EcoRI DNA fragment from plasmid pMON13304. The
 following genetic elements are derived from pMON13332;
 beta-lactamase gene (AMP), pBR327 origin of
 replication, phage fl origin of replication as the
 10 transcription terminator, recA promoter, g10L ribosome
 binding site and the bases encoding amino acids 15-69
 and 106-125 of the (15-125) hIL-3 gene with the
 following change, 42D, 45M, 46S, and 116W. The bases
 encoding amino acids 70-105 of the (15-125) gene with
 15 the following change, 98I and 100R are derived from
 pMON13304. The resulting plasmid, pMON13329, encodes
 the (15-125) hIL-3 variant with the following amino
 acid sequence:

20 Peptide # A8 [SEQ ID NO:406]

EXAMPLE 67

Construction of pMON5853 (Fig 6) which encodes [Met-
 25 (15-133)hIL-3(Arg129)]

Plasmid DNA of pMON5847 (Example 2) was treated
 with NcoI. The restriction enzyme was inactivated by
 heat treatment (65°C for 10 minutes). The DNA was then
 30 treated with large fragment of DNA polymerase I
 (Klenow) in the presence of all four nucleotide
 precursors. This produces DNA termini with non-
 overlapping ends. After 5 minutes at 37°C, the
 polymerase was inactivated by heat treatment at 65°C
 35 for 10 minutes. The DNA was then treated with HpaI, an
 enzyme which produces non-overlapping termini. The DNA

was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis.

- 5 A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

10

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:400]
M N C S N [SEQ ID NO:401]

- 15 The lower line contains the one-letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and asparagine.

- 20 When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

- 25 The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg129) which has the following amino acid sequence:

- Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
30 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
35 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg

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	60	65	70
	Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu		
	75	80	85
	Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly		
5	90	95	100
	Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr		
	105	110	115
	Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:407]		
	120	125	

10

DNA sequence #A10 pMON13252 50D

ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGGT GAAGACCAAG
15 ATATCCTGAT GGAACAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA ACTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
20 [SEQ ID NO:408]

Examples 69-76

The variants in Table 5 were constructed by cassette
25 mutagenesis using methods described in the Materials
and Methods and the Examples contained herein,
particularly Examples 54-58 . Parental plasmid DNA
(Table 5), digested with the appropriate restriction
enzymes (Table 5), was ligated with the indicated
30 annealed pairs of complementary oligonucleotides (Table
5). The assembled oligonucleotides create appropriate
restriction ends and a portion of the (15-125) hIL-3
gene sequence (pMON13288 [SEQ ID NO:100]). Individual
isolates were screened by restriction analysis and DNA
35 sequenced to confirm that the desired changes in the
(15-125) hIL-3 variant gene were made. The

oligonucleotides create change(s) in the (15-125) hIL-3 gene which encode the corresponding amino acid substitution(s) in the variant polypeptide (Table 5). The amino acids substitutions in addition to and/or
5 different from those in polypeptide # 25 [SEQ ID NO:89] are indicated in Table 5. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and
10 the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 5 is shown in Table 1.

Examples 77-82

15 The variants in Table 6 were constructed by methods described in the Materials and Methods and the Examples contained herein, particularly in Examples 60 and 61. Parental plasmid DNA (Table 6), digested with the
20 appropriate restriction enzymes (Table 6), was ligated with the indicated restriction fragment (Table 6). Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made.
25 The resulting mutated (15-125) hIL-3 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 6). The amino acids substitutions in addition to and/or different from those in polypeptide # 25 [SEQ ID NO:89] are indicated in Table
30 6. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells)
35 for some of the variants in Table 6 is shown in Table 1.

Example 83

Construction of pMON13368

5 One of the DNA fragments to construct the plasmid, pMON13368, was generated by site-directed mutagenesis employing PCR techniques described in the Materials and Methods and the Examples contained herein, particularly Example 53. The template for the PCR reaction was plasmid, pMON13289, DNA using the oligonucleotides, Oligo #B13 18I23A25H [SEQ ID NO: 182] and Oligo #B14 2341HIN3 [SEQ ID NO:183], as primers. The resulting DNA product was digested with the restriction enzymes NcoI and HindIII. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The restriction fragment was purified by phenol/chloroform extraction and precipitation with equal volume isopropanol in the presence of 2M NH₄OAc. The oligonucleotide, Oligo #B13 18I23A25H [SEQ ID NO:182], changes the codon at position 23 of (15-125) hIL-3 variant gene pMON13289 [SEQ ID NO:103] from 'ATT' to 'GCA' (Ile to Ala). The 3619 base pair NcoI, HindIII restriction fragment from pMON2341 was ligated to the PCR-generated NcoI, HindIII restriction fragment. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The plasmid, pMON13368, contains the (15-125) hIL-3 variant gene (DNA sequence #B15 [SEQ ID NO:346]) which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B15 [SEQ ID NO.:278]

35

Example 84

Construction of pMON13380

Plasmid, pMON13368, DNA was digested with restriction
 5 enzymes EcoRI and HindIII. The resulting 3900 base pair
 EcoRI,HindIII fragment contains the following genetic
 elements; beta-lactamase gene (AMP), pBR327 origin of
 replication, phage F1 origin of replication as the
 transcription terminator, precA promoter, gl0L ribosome
 10 binding site and the DNA sequence encoding amino acids
 15-105 of the variant pMON13368. The 3900 base pair
 EcoRI,HindIII restriction fragment from pMON13368 was
 ligated to the following annealed complementary
 oligonucleotides.

15
 Oligo # B48 9E12Q6V1 [SEQ ID NO:217]
 Oligo # B49 9E12Q6V3 [SEQ ID NO:218]

 Oligo #49 120Q123E2 [SEQ ID NO:63]
 20 Oligo #50 120Q123E4 [SEQ ID NO:64]

When assembled, the oligonucleotides create EcoRI and
 HindIII restriction ends and the DNA sequence that
 encodes amino acids 106-125 of (15-125) hIL-3 with the
 25 following amino acid substitution; 109E, 112Q, 116V,
 120Q and 123E. The codons used in the (15-125) hIL-3
 gene are those found in the hIL-3 cDNA sequence except
 at those positions where amino acid substitutions were
 made. Individual isolates were screened by restriction
 30 analysis and DNA sequenced to confirm that the desired
 changes in the (15-125) hIL-3 variant gene were made.
 The plasmid, pMON13380, contains the (15-125) hIL-3
 variant gene (DNA sequence #B16 [SEQ ID NO:347]) which
 encodes the (15-125) hIL-3 variant polypeptide with the
 35 following amino acid sequence:

Polypeptide #B16 [SEQ ID NO.:279]

Example 85

5 Construction of pMON13476

One of the DNA fragments to construct the plasmid, pMON13476, was generated by site-directed mutagenesis employing PCR techniques described in the Materials and
 10 Methods and the Examples contained herein, particularly Example 54. The template for the PCR reaction was plasmid, pMON13287, DNA using the oligonucleotides, Oligo #B13 18I23A25H [SEQ ID NO:182] and Oligo #B14 2341HIN3 [SEQ ID NO.:183] as primers.
 15 The resulting DNA product was digested with the restriction enzymes NcoI and HindIII. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The restriction fragment was purified by phenol/chloroform extraction and
 20 precipitation with equal volume isopropanol in the presence of 2M NH4OAc. The oligonucleotide, Oligo #B13 18I23A25H [SEQ ID NO.:182], changes the codon at position 23 of (15-125) hIL-3 variant gene, pMON13287, [SEQ ID NO:97] from 'ATT' to 'GCA' (Ile to Ala). The
 25 3619 base pair NcoI, HindIII restriction fragment from pMON2341 was ligated to the PCR-generated NcoI, HindIII restriction fragment. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant
 30 gene were made. The resulting clone also contained a change, that was not designed in the mutagenic oligonucleotide, which changed the codon at position -1 from 'GCT' to 'GAT' which changes the amino acid from Alanine to Aspartic Acid. The plasmid, pMON13476,
 35 contains the (15-125) hIL-3 variant gene (DNA sequence #B52 [SEQ ID NO:303]) which encodes the (15-125) hIL-3

variant polypeptide with the following amino acid sequence:

Polypeptide #B52 [SEQ ID NO.:314]

5

Examples 86-92

The variants in Table 7 were constructed by PCR techniques using methods described in the Materials and Methods and the Example contained herein, particularly Example 51. Two sequential PCR reactions were used to create the variants. In the first PCR reaction pMON13287 plasmid DNA served as the template and the two oligonucleotides indicated in Table 7 served as the primers. Following the PCR extension reaction, the PCR product was partially purified to remove primer that was not extended. In the second PCR reaction pMON13287 plasmid DNA served as the template, the purified PCR product from the first PCR reaction served as one of the primers and the Oligo #B14 2341Hin3 [SEQ ID NO:183] as the second primer. The product from the second PCR reaction was partially purified and digested with restriction enzymes NcoI and HindIII and ligated with the 3619 base pair NcoI,HindIII fragment from pMON2341. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The amino acids substitutions in addition to and/or different from those in polypeptide # 24 [SEQ ID NO:88] are indicated in Table 7. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 7 is shown in Table 1.

Examples 93-120

The variants in Table 8 were constructed by cassette
 5 mutagenesis using methods described in the Materials
 and Methods and the Examples contained here,
 particularly Examples 54-58. Parental plasmid DNA
 (Table 8), digested with the appropriate restriction
 enzymes (Table 8), was ligated with the indicated
 10 annealed pairs of complementary oligonucleotides (Table
 8). The assembled oligonucleotides create the
 appropriate restriction ends and a portion of (15-125)
 hIL-3 gene (pMON13288 [SEQ ID NO:100]) sequence. The
 oligonucleotides create change(s) in the (15-125) hIL-3
 15 variant gene which encode the corresponding amino acid
 substitution(s); and/or deletions from the C-terminus
 of the variant polypeptide (Table 8). Individual
 isolates were screened by restriction analysis and DNA
 sequenced to confirm that the desired changes in the
 20 (15-125) hIL-3 variant gene were made. The amino acids
 substitutions in addition to and/or different from
 those in polypeptide # 25 [SEQ ID NO:88] are indicated
 in Table 8. The table also shows the plasmid
 designation (pMON number), DNA sequence identification
 25 number for the mutated hIL-3 gene and the
 identification number for the the resulting variant
 polypeptide. The biological activity (growth promoting
 activity in AML 193 cells) for some of the variants in
 Table 5 is shown in Table 1.

30

Example 121

Construction of pMON13446

Plasmid, pMON13287, DNA (purified from the E. coli
 35 strain GM48 {dam-}) was digested with restriction
 enzymes NcoI and ClaI. The resulting 3942 base pair
 NcoI, ClaI fragment contains the following genetic

elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, precA promoter, gl0L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant pMON13287. The 3942 base pair NcoI,ClaI restriction fragment from pMON13368 was ligated to the following annealed complementary oligonucleotides.

10 Oligo #B57 338UP [SEQ ID NO:226]

Oligo #B56 338DOWN [SEQ ID NO:225]

When assembled, the oligonucleotides create NcoI and ClaI restriction ends and the DNA sequence that encodes the following 14 amino acid sequence; Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403] and the DNA sequence which encodes amino acids 15-20 of the (15-125) hIL-3 variant gene, pMON13287 [SEQ ID NO:97]. The resulting variant polypeptide has a 14 amino acid N-terminal extension fused to the (15-125) hIL-3 variant polypeptide, pMON13288 [SEQ ID NO: 88]. The plasmid, pMON13446, contains the (15-125) hIL-3 variant gene (DNA sequence #B53 [SEQ ID NO:404]) which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B53 [SEQ ID NO.:315]

30 Example B54

Construction of pMON13390

Plasmid, pMON13288, DNA (purified from the E. coli strain GM48 {dam-}) was digested with restriction enzymes NcoI and ClaI. The resulting 3942 base pair NcoI,ClaI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the

transcription terminator, precA promoter, g10L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant pMON13288. The 3942 base pair NcoI, ClaI restriction fragment from pMON13288 was ligated to the following annealed complementary oligonucleotides.

	Oligo #B57	338UP	[SEQ ID NO:226]
10	Oligo #B56	338DOWN	[SEQ ID NO:225]

When assembled, the oligonucleotides create NcoI and ClaI restriction ends and the DNA sequence which encodes the following 14 amino acid sequence; Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403] and the DNA sequence which encodes amino acids 15-20 of the (15-125) hIL-3 variant gene pMON13288 [SEQ ID NO:100]. The resulting variant has a 14 amino acid N-terminal extension fused to the (15-125) hIL-3 variant polypeptide, pMON13288 [SEQ ID NO:88]. The plasmid, pMON13390, contains the (15-125) hIL-3 variant gene (DNA sequence #B54 [SEQ ID NO.:405] which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B54 [SEQ ID NO:316]

Examples 133-136

The variants in Table 10 were constructed by methods described in Materials and Methods and in Examples contained herein, particularly Examples 54-58. Parental plasmid DNA (Table 10), digested with the appropriate restriction enzymes (Table 10) was ligated with the indicated restriction fragment containing the changes listed (Table 10). The resulting mutated (15-125) IL-3 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 10). The amino acid

substitutions in addition to and/or different from those in polypeptide #25 [SEQ ID NO: 89] are indicated in Table 10. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 10 is shown in Table 1.

Examples 123-132

The variants in Table 9 were constructed by cassette mutagenesis using methods described in Materials and Methods and in Examples 54-58 contained herein. Parental plasmid DNA (Table 9), digested with the appropriate restriction enzymes (Table 9), was ligated with the indicated annealed pairs of complementary oligonucleotides (Table 9). The assembled oligonucleotides create the appropriate restriction fragment which was inserted into the (15-125) hIL-3 gene (pMON13288 [SEQ ID NO:100] between these restriction sites. The deletions or substitutions encoded by the oligonucleotide in the (15-125) IL-3 gene correspond to the amino acid deletions or substitutions in the variant polypeptide (Table 9). The amino acid substitutions or deletions, in addition to and/or different from those in the polypeptide #25 [SEQ ID NO:89] are indicated in Table 9. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 9 is shown in Table 1.

Formula XI shown below is a representation of a [(15-125)hIL-3 mutein] with numbers in bold type added above the amino acids to represent the position at which the amino acid below the bolded number appears in native (1-133)hIL-3 [e. g. the amino acid at position 1 of Formula XI corresponds to the Asn which appears at position 15 in native (1-133)hIL-3]. The number shown in bold indicates the amino acids that correspond to

5	<div> <div>15</div> <div>20</div> <div>25</div> </div> <div> Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln 1 5 10 15 </div>															
10	<div> <div>30</div> <div>35</div> <div>40</div> </div> <div> Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp 20 25 30 </div>															
15	<div> <div>45</div> <div>50</div> <div>55</div> </div> <div> Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu 35 40 45 </div>															
20	<div> <div>60</div> <div>65</div> <div>70</div> </div> <div> Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile 50 55 60 </div>															
25	<div> <div>75</div> <div>80</div> <div>85</div> </div> <div> Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr 65 70 75 </div>															
30	<div> <div>90</div> <div>95</div> <div>100</div> </div> <div> Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp 80 85 90 </div>															
35	<div> <div>105</div> <div>110</div> <div>115</div> </div> <div> Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu 95 100 105 </div> <div> <div>120</div> <div>125</div> </div> <div> Asn Ala Gln Ala Gln Gln [SEQ ID NO:23] 110 </div>															

Table 5

Example	pMON number	Parental plasmid/ restriction digest	oligo pair 1,4	oligo pair 2,5	oligo pair 3,6	amino acid changes	resulting polypeptide
Example 69	pMON13406 SEQ ID NO:332	pMON13288/ NcoI, EcoRV	19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26	19Ala	polypeptide B1 SEQ ID NO:264
Example 70	pMON13414 SEQ ID NO:333	pMON13288/ NcoI, EcoRV	19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26	19Ile	polypeptide B2 SEQ ID NO:265
Example 71	pMON13407 SEQ ID NO:334	pMON13288/ NcoI, EcoRV	18I25H1 OLIGO# 1 SEQ ID NO:15 18I25H4 OLIGO# 2 SEQ ID NO:16	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181	45Val	polypeptide B3 SEQ ID NO:266
Example 72	pMON13405 SEQ ID NO:335	pMON13288/ NcoI, EcoRV	19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181	19Ala,45Val	polypeptide B4 SEQ ID NO:267
Example 73	pMON13415 SEQ ID NO:336	pMON13288/ NcoI, EcoRV	19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181	19Ile,45Val	polypeptide B5 SEQ ID NO:268
Example 74	pMON13408 SEQ ID NO:337	pMON13288/ EcoRV, NsiI	49Ile1 OLIGO# B7 SEQ ID NO:176 49Ile4 OLIGO# B8 SEQ ID NO:177	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Ile	polypeptide B6 SEQ ID NO:269

Table 5 cont

Example 75	pMON13409 SEQ ID NO:338	pMON13288/ EcoRV, NsiI	49Leu1 SEQ ID NO:178 OLIGO# B9 49Leu4 OLIGO# B10 SEQ ID NO:179	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Leu	polypeptide B7 SEQ ID NO:270
Example 76	pMON13410 SEQ ID NO:339	pMON13288/ EcoRV, NsiI	49Asp1 OLIGO# B5 SEQ ID NO:174 49Asp4 OLIGO# B6 SEQ ID NO:175	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Asp	polypeptide B8 SEQ ID NO:271

Table 6

Example No	plasmid pMON number	Parental plasmid/ restriction digest	restriction fragment	amino acid substitutions	resulting polypeptide
Example 77	pMON13422 SEQ ID NO:340	pMON13408/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Ple	polypeptide B9 SEQ ID NO:272
Example 78	pMON13423 SEQ ID NO:341	pMON13408/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Ple	polypeptide B10 SEQ ID NO:273
Example 79	pMON13424 SEQ ID NO:342	pMON13409/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Leu	polypeptide B11 SEQ ID NO:274
Example 80	pMON13425 SEQ ID NO:343	pMON13409/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Leu	polypeptide B12 SEQ ID NO:275
Example 81	pMON13426 SEQ ID NO:344	pMON13410/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Asp	polypeptide B13 SEQ ID NO:276
Example 82	pMON13429 SEQ ID NO:345	pMON13410/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Asp	polypeptide B14 SEQ ID NO:277

Table 7

Example	MON number	template	Step one PCR primer1	Step one PCR primer2	Step two PCR primer1 product from step one	Step two PCR primer2 OLIGO# B14 SEQ ID NO:183	Amino Acid Substitutions	Polypeptide
Example 86	pMON13475 SEQ ID NO:348	pMON13287	1823A25H OLIGO# B13 SEQ ID NO:182	42D45V46S50D OLIGO# B19 SEQ ID NO:188	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	42D,46S,50D	Polypeptide # B17 SEQ ID NO 280
Example 87	pMON13366 SEQ ID NO:349	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45V46S50D OLIGO# B19 SEQ ID NO:188	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	42N,46S,50D	Polypeptide # B18 SEQ ID NO 281
Example 88	pMON13367 SEQ ID NO:350	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42A45V46S50D OLIGO# B17 SEQ ID NO:186	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	46S,50D	Polypeptide # B19 SEQ ID NO 282
Example 89	pMON13369 SEQ ID NO:351	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45V46S50D OLIGO# B21 SEQ ID NO:190	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	42D,46S,50D	Polypeptide # B20 SEQ ID NO 283
Example 90	pMON13370 SEQ ID NO:352	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42A45M46S50D OLIGO# B16 SEQ ID NO:185	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	45M,46S,50D	Polypeptide # B21 SEQ ID NO 284
Example 91	pMON13373 SEQ ID NO:353	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45M46S50D OLIGO# B18 SEQ ID NO:187	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	42D,45M,46S 50D	Polypeptide # B22 SEQ ID NO 285
Example 92	pMON13374 SEQ ID NO:354	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42S45M46S50D OLIGO# B20 SEQ ID NO:189	product from step one	2341HN3 OLIGO# B14 SEQ ID NO:183	42S,45M46S 50D	Polypeptide # B23 SEQ ID NO 286

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Table 8

Example	plasmid	parental plasmid	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	resulting amino acid sub[se].	polypeptide
Example 93	PMON13375 SEQ ID NO:355	PMON13287/ EcoRI, HindIII	S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 OLIGO# B51 SEQ ID NO:220 S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 OLIGO# B55 SEQ ID NO:224	S116VDJ1 OLIGO# B52 SEQ ID NO:221 SECRIDJ3 OLIGO# B53 SEQ ID NO:222				15-119	polypeptide B24 SEQ ID NO:287
Example 94	PMON13376 SEQ ID NO:356	PMON13476/ EcoRI, HindIII	S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 OLIGO# B55 SEQ ID NO:224	S116VDJ1 OLIGO# B52 SEQ ID NO:221 SECRIDJ3 OLIGO# B53 SEQ ID NO:222				15-119, 23A, 112Q	polypeptide B25 SEQ ID NO:288
Example 95	PMON13377 SEQ ID NO:357	PMON13475/ EcoRI, HindIII	S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 OLIGO# B55 SEQ ID NO:224	S116VDJ1 OLIGO# B52 SEQ ID NO:221 SECRIDJ3 OLIGO# B53 SEQ ID NO:222				15-119, 23A, 42D, 46S, 50D, 112Q	polypeptide B26 SEQ ID NO:289
Example 96	PMON13378 SEQ ID NO:358	PMON13365/ EcoRI, HindIII	S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 OLIGO# B51 SEQ ID NO:220	S116VDJ1 OLIGO# B52 SEQ ID NO:221 SECRIDJ3 OLIGO# B53 SEQ ID NO:222				15-119, 23A	polypeptide B27 SEQ ID NO:290
Example 97	PMON13379 SEQ ID NO:359	PMON13367/ EcoRI, HindIII	9E12Q6V1 OLIGO# B48 SEQ ID NO:217 9E12Q6V3 OLIGO# B49 SEQ ID NO:218	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64				46S, 50D, 112Q	polypeptide B28 SEQ ID NO:291
Example 98	PMON13385 SEQ ID NO:360	PMON13287/ NcoI, EcoRV	18125W1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	42A45V3 9 SEQ ID NO:23 42A45V6 OLIGO# 10 SEQ ID NO:24			29V, 32R, 34S	polypeptide B29 SEQ ID NO:292
Example 99	PMON13381 SEQ ID NO:361	PMON13287/ NcoI, EcoRI	73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	101A105Q4 OLIGO# 43 SEQ ID NO:57 101A105Q8 OLIGO# 44 SEQ ID NO:58			82W	polypeptide B30 SEQ ID NO:293

Table 8 cont

Example 100	PMONI3383 SEQ ID NO:362	PMONI3475/ EcoRI, HindIII	9E1206V1 OLIGO1 B48 SEQ ID NO:217 9E1206V5 OLIGO1 B49 SEQ ID NO:218	120Q13E2 OLIGO1 49 SEQ ID NO:63 120Q13E4 OLIGO1 50 SEQ ID NO:64			23A, 42D, 46S, 50D 1120	polypeptide B31 SEQ ID NO:294
Example 101	PMONI3384 SEQ ID NO:363	PMONI3287/ EcoRI, HindIII	9E1206V1 OLIGO1 B48 SEQ ID NO:217 9E1206V5 OLIGO1 B49 SEQ ID NO:218	120Q13E2 OLIGO1 49 SEQ ID NO:63 120Q13E4 OLIGO1 50 SEQ ID NO:64			1120	polypeptide B32 SEQ ID NO:295
Example 102	PMONI3388 SEQ ID NO:364	PMONI3287/ EcoRV, NotI	50D56S1 OLIGO1 B42 SEQ ID NO:211 50A5P4 OLIGO1 B41 SEQ ID NO:210	60S62V2 OLIGO1 27 SEQ ID NO:41 46SER5 OLIGO1 B43 SEQ ID NO:212	67N69E3 OLIGO1 31 SEQ ID NO:45 67N69E6 OLIGO1 32 SEQ ID NO:46		50D, 56S	polypeptide B33 SEQ ID NO:296
Example 103	PMONI3389 SEQ ID NO:365	PMONI3287/ NotI, EcoRV	18I25H1 OLIGO1 SEQ ID NO:15 18I25H4 OLIGO1 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45M3 OLIGO1 B32 SEQ ID NO:201 42D45M6 OLIGO1 B33 SEQ ID NO:202		42D, 45M	polypeptide B34 SEQ ID NO:297
Example 104	PMONI3391 SEQ ID NO:366	PMONI3287/ NotI, EcoRV	18I25H1 OLIGO1 SEQ ID NO:15 18I25H4 OLIGO1 SEQ ID NO:16	34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200	42A45V3 OLIGO1 9 SEQ ID NO:203 42A45V6 OLIGO1 10 SEQ ID NO:204		34S	polypeptide B35 SEQ ID NO:298
Example 105	PMONI3392 SEQ ID NO:367	PMONI3287/ NotI, EcoRV	18I25H1 OLIGO1 SEQ ID NO:15 18I25H4 OLIGO1 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45V3 OLIGO1 B34 SEQ ID NO:203 42D45V6 OLIGO1 B35 SEQ ID NO:204		42D	polypeptide B36 SEQ ID NO:299
Example 106	PMONI3393 SEQ ID NO:368	PMONI3287/ NotI, EcoRV	23A1A1 OLIGO1 B36 SEQ ID NO:195 23A1A4 OLIGO1 B37 SEQ ID NO:196	34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200	42D45M46S3 OLIGO1 B36 SEQ ID NO:205 42D45M46S6 OLIGO1 B37 SEQ ID NO:206		23A, 34S, 42D, 45M 46S	polypeptide B37 SEQ ID NO:300

Table 8 cont

Example 107	PHONI3394 SEQ ID NO:369	PHONI3287/ NcoI, EcoRV	18125H1 OLIGO1 SEQ ID NO:15 18125H4 OLIGO2 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45M46S3 OLIGO1 B36 SEQ ID NO:205 42D45M46S6 OLIGO1 B37 SEQ ID NO:206	42D, 45M, 46S	polypeptide B38 SEQ ID NO:301
Example 108	PHONI3395 SEQ ID NO:370	PHONI3287/ NcoI, EcoRV	23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196	29V32R34S2 OLIGO1 B28 SEQ ID NO:197 29V32R34S5 OLIGO1 B29 SEQ ID NO:198	42D45V46S3 OLIGO1 B38 SEQ ID NO:207 42D45V46S6 OLIGO1 B39 SEQ ID NO:208	23A, 29V, 32R, 34S 42D, 46S	polypeptide B39 SEQ ID NO:302
Example 109	PHONI3396 SEQ ID NO:371	PHONI3287/ NsiI, EcoRI	73G76A1 OLIGO1 41 SEQ ID NO:55 73G76A4 OLIGO1 42 SEQ ID NO:56	79R82Q2 OLIGO1 39 SEQ ID NO:53 79R82Q5 OLIGO1 40 SEQ ID NO:54	100ARG3 SEQ ID NO:1 87S93S98I7 OLIGO1 36 SEQ ID NO:50	100R, 101M	polypeptide B40 SEQ ID NO:303
Example 110	PHONI3397 SEQ ID NO:372	PHONI3287/ NsiI, EcoRI	73G76A1 OLIGO1 41 SEQ ID NO:55 73G76A4 OLIGO1 42 SEQ ID NO:56	82TRP2 OLIGO1 B44 SEQ ID NO:213 82TRP5 OLIGO1 B45 SEQ ID NO:214	100ARG3 OLIGO1 B22 SEQ ID NO:191 87S93S98I7 OLIGO1 36 SEQ ID NO:50	82W, 100R, 101M	polypeptide B41 SEQ ID NO:304
Example 111	PHONI3398 SEQ ID NO:373	PHONI3287/ NcoI, EcoRV	18125H1 OLIGO1 SEQ ID NO:15 18125H4 OLIGO2 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45V46S3 OLIGO1 B38 SEQ ID NO:207 42D45V46S6 OLIGO1 B39 SEQ ID NO:208	42D, 46S	polypeptide B42 SEQ ID NO:305
Example 112	PHONI3399 SEQ ID NO:374	PHONI3388/ NcoI, EcoRV	23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196	29V32R34S2 OLIGO1 B28 SEQ ID NO:197 29V32R34S5 OLIGO1 B29 SEQ ID NO:198	42D45V46S3 OLIGO1 B38 SEQ ID NO:207 42D45V46S6 OLIGO1 B39 SEQ ID NO:208	23A, 29V, 32R, 34S 42D, 46S	polypeptide B43 SEQ ID NO:306
Example 113	PHONI3404 SEQ ID NO:375	PHONI3287/ EcoRI, HindIII	59E2Q6V1 OLIGO1 B54 SEQ ID NO:223 59E2Q6V3 OLIGO1 B55 SEQ ID NO:224	5116VDJ1 OLIGO1 B52 SEQ ID NO:221 59E2Q6V3 OLIGO1 53 SEQ ID NO:222		15-119 112Q	polypeptide B44 SEQ ID NO:307

Table 8 cont

Example 114	PHONI3387 SEQ ID NO:376	PHONI3387/ EcoRV, NsiI	50ASP1 OLIGO1 B40 SEQ ID NO:209 50ASP4 OLIGO1 B41 SEQ ID NO:210	60S62V2 OLIGO1 27 SEQ ID NO:41 60S62V5 OLIGO1 28 SEQ ID NO:42	67N69E3 OLIGO1 31 SEQ ID NO:45 67N69E6 OLIGO1 32 SEQ ID NO:46	50D	polypeptide B45 SEQ ID NO:308
Example 115	PHONI3416 SEQ ID NO:377	PHONI3387/ NcoI/EcoRV	1812SH1 OLIGO1 3 SEQ ID NO:15 1812SH4 OLIGO1 4 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45H46S3 OLIGO1 B38 SEQ ID NO:207 42D45H46S6 OLIGO1 B39 SEQ ID NO:208	42D, 46S, 50D	polypeptide B46 SEQ ID NO:309
Example 116	PHONI3417 SEQ ID NO:378	PHONI3387/ NcoI/EcoRV	1812SH1 OLIGO1 3 SEQ ID NO:15 1812SH4 OLIGO1 4 SEQ ID NO:16	29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18	42D45H46S3 OLIGO1 B36 SEQ ID NO:205 42D45H46S6 OLIGO1 B37 SEQ ID NO:206	42D, 45H, 46S, 50D	polypeptide B47 SEQ ID NO:310
Example 117	PHONI3420 SEQ ID NO:379	PHONI3388/ NcoI, EcoRV	23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196	34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200	42D45H46S3 OLIGO1 B38 SEQ ID NO:207 42D45H46S6 OLIGO1 B39 SEQ ID NO:208	23A, 34S, 42D, 46S 50D, 56S	polypeptide B48 SEQ ID NO:311
Example 118	PHONI3421 SEQ ID NO:380	PHONI3388/ NcoI, EcoRV	23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196	34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200	42D45H46S3 OLIGO1 B36 SEQ ID NO:205 42D45H46S6 OLIGO1 B37 SEQ ID NO:206	23A, 34S, 42D, 45H 46S, 50D, 56S	polypeptide B49 SEQ ID NO:311
Example 119	PHONI3432 SEQ ID NO:381	PHONI3387/ NcoI, EcoRV	23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196	34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200	42D45H46S3 OLIGO1 B36 SEQ ID NO:205 42D45H46S6 OLIGO1 B37 SEQ ID NO:206	23A, 34S, 42D, 45H 46S, 50D	polypeptide B50 SEQ ID NO:312
Example 120	PHONI3382 SEQ ID NO:382	PHONI3387/ EcoRI, HindIII	9E12Q6W1 OLIGO1 B46 SEQ ID NO:215 9E12Q16W3 OLIGO1 B47 SEQ ID NO:216	120Q123E2 OLIGO1 49 SEQ ID NO:63 120Q123E4 OLIGO1 50 SEQ ID NO:64		112Q, 116W	polypeptide B51 SEQ ID NO:313

Table 9

Example No.	Plasmid	Parental Plasmid/ Restriction Digest	Oligo pair	Oligo pair	Oligo pair	Amino acid changes	Polypeptide
Example 124	pMON13400 SEQ ID NO:384	pMON13288 Restriction NcoI-EcoRV	20P23A1 SEQ ID NO:232 20P23A4 SEQ ID NO:233	2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	20P 23A 291 34S 37S 38A 45V 46S	Polypeptide C-2 SEQ ID NO:317
Example 125	pMON13402 SEQ ID NO:385	pMON13288 Restriction NcoI-EcoRV	23L1 SEQ ID NO:234 23L4 SEQ ID NO:235	2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	23L 291 34S 37S 38A 45V 46S	Polypeptide C-3 SEQ ID NO:318
Example 131	pMON13440 SEQ ID NO:386	pMON13288 Restriction NcoI-EcoRV	1813A5H1 SEQ ID NO:195 1813A5H4 SEQ ID NO:196	2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	181 23A 25H 291 34S 37S 38A 45V 46S	Polypeptide C-10 SEQ ID NO:319
Example 132	pMON13451 SEQ ID NO:387	pMON13288 Restriction NcoI-EcoRV	1910L3A1 SEQ ID NO:230 1910L3A4 SEQ ID NO:231	2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	191 20L 23A 291 34S 37S 38A 45V 46S	Polypeptide C-11 SEQ ID NO:320
Example 130	pMON13419 SEQ ID NO:388	pMON13288 Restriction EcoRV-NsiI	50D61S1 SEQ ID NO:240 50D61S4 SEQ ID NO:241	62P3H5S2 SEQ ID NO:244 62P3H5 SEQ ID NO:246	67Q3 SEQ ID NO:248 65S67Q6 SEQ ID NO:247	50D 61S 62P 63H 65S 67Q	Polypeptide C-8 SEQ ID NO:325
Example 126	pMON13403 SEQ ID NO:388	pMON13288 Restriction EcoRV-NsiI	50D61S1 SEQ ID NO:240 50D61S4 SEQ ID NO:241	62P3H2 SEQ ID NO:245 62P3H5 SEQ ID NO:246	67Q3 SEQ ID NO:248 67Q6 SEQ ID NO:249	50D 61S 62P 63H 67Q	Polypeptide C-4 SEQ ID NO:321

Table 9 cont

Example 123	pMON13418 SEQ ID NO:393	pMON13288 Restriction NotI-EcoRI	76P1 SEQ ID NO:250 76P5 SEQ ID NO:251	78S2 SEQ ID NO:252 78S6 SEQ ID NO:253	6VYWPTT3 SEQ ID NO:242 6VYWPTT7 SEQ ID NO:243	101A105Q4 SEQ ID NO:57 101A105Q8 SEQ ID NO:58	76P 78S 85V 87Y 88W 91P 95T 98T 109L 112Q 116S	Polypeptide C-1 SEQ ID NO:326
Example 127	pMON13411 SEQ ID NO:390	pMON13288 Restriction EcoRI-HindIII	09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228	120Q123E2 SEQ ID NO:63 120Q123E4 SEQ ID NO:64				Polypeptide C-5 SEQ ID NO:322
Example 128	pMON13412 SEQ ID NO:391	pMON13288 Restriction EcoRI-HindIII	9LQ6S1181 Seq ID NO:255 9LQ6S1183 SEQ ID NO:256				15-118 109L 112Q 116S	Polypeptide C-6 SEQ ID NO:323
Example 129	pMON13413 SEQ ID NO:392	pMON13288 Restriction EcoRI-HindIII	09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228	117S2 SEQ ID NO:229 120Q123E4 SEQ ID NO:64			109L 112Q 116S 117S	Polypeptide C-7 SEQ ID NO:324

Table 10

Example No	Plasmid	Parental plasmid/ Restriction digest	Restriction fragment	Amino Acid changes	Polypeptide
Example 133	pMON13428 SEQ ID NO:394	pMON13411 NsiI-EcoRI	102 bp NsiI-EcoRI fragment from pMON13418	76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S	Polypeptide C-9 SEQ ID NO:327
Example 134	pMON13459 SEQ ID NO:395	pMON13428 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S	Polypeptide C-12 SEQ ID NO:328
Example 135	pMON13467 SEQ ID NO:396	pMON13413 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 109L 112Q 116S 109L 112Q 116S 117S	Polypeptide C-13 SEQ ID NO:329
Example 136	pMON13492 SEQ ID NO:397	pMON13418 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T	Polypeptide C-14 SEQ ID NO:330